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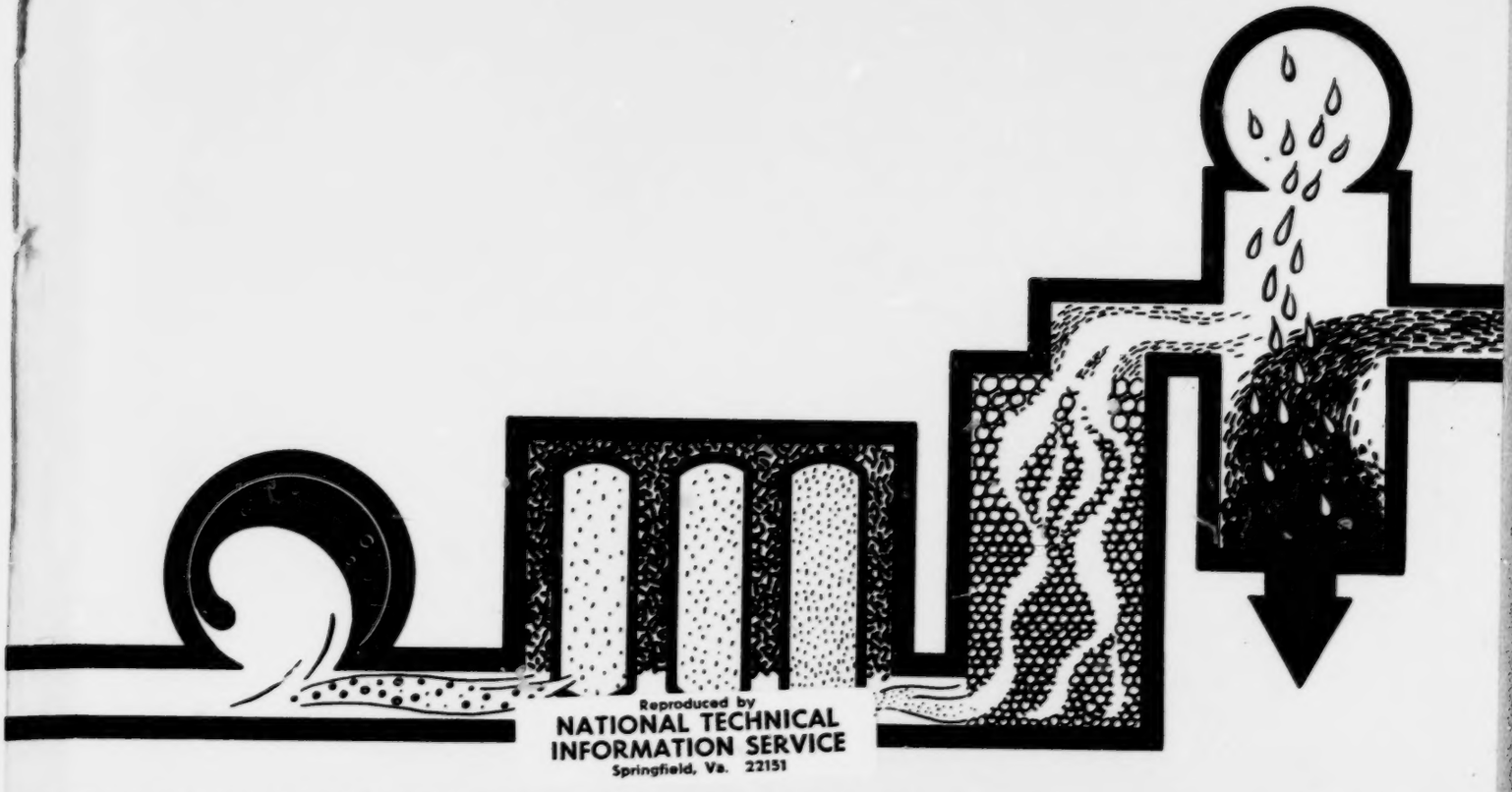
A STUDY OF NITRIFICATION AND DENITRIFICATION



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A STUDY OF NITRIFICATION AND DENITRIFICATION

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for the

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ABSTRACT

A program to incorporate biological denitrification into a wastewater treatment system was undertaken with the objective of developing a process that depended exclusively on the carbon compounds contained in the wastewater to supply metabolic energy to the microflora. A unique treatment system incorporating a large microbial biomass supported on submerged plastic rings was utilized as the laboratory device. In the experimental program the incoming nitrogenous material was oxidized to nitrate in an aerobic phase and reduced to nitrogen gas in an anaerobic phase. This system accomplished a complete wastewater treatment - carbon oxidation in the aerobic phase, nitrogen removal in the anaerobic phase.

The conditions for developing a nitrifying microflora were investigated using a primary wastewater effluent as feed. The flow into the system was varied to give a range of residence times, and the effluents from the system were analyzed to determine the degree of oxidation. Anaerobic batch experiments were carried out to determine if aerobic reserves could support denitrification. These studies demonstrated that under appropriate conditions almost 100% of the nitrates could be reduced.

The final phase of the program was to couple the aerobic and anaerobic stages. The effluent from the aerobic unit served as the feed for the anaerobic process. At appropriate intervals this situation was reversed by switching the airflows and feed sources. Over 95% of the wastewater nitrogen in wastewater was removed. Nitrate-nitrogen removal rates ranged from 0.600 to 1.00 mg/hr/g MLVS. A mathematical model was developed which described the response to cycled aerobic-anaerobic operation. The alternating cycle approach was shown to be an effective method for removing nitrogen from wastewater.

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I. CONCLUSIONS

SIGNIFICANT FINDINGS OF THE PRESENT PROGRAM

This program achieved its initial objectives of demonstrating that biological denitrification can take place with microbial stored food reserves serving as the energy source for the process. A denitrification process was developed that operated on a continuous feed of primary effluent. The wastewater was carried through a complete secondary treatment and discharged as a well oxidized and denitrified polished effluent. The system includes a practical method of cycling the microflora between aerobic and anaerobic modes without interrupting continuous flow. The overall study was aimed at identifying and evaluating some of the parameters that influence the systems efficiency and at developing the basic information that would permit utilization of the process on an enlarged scale. Several of the more significant findings of this program are presented.

Efficient nitrification must always precede denitrification to achieve high overall nitrogen removal. The proportion of the incoming nitrogen oxidized to nitrate is strongly influenced by the organic loads. There is an optimum loading of 1.1 g COD/day / g MLVS which results in practically all of the incoming nitrogen being converted to nitrate nitrogen.

The rate of oxygen uptake (Q_{O_2}) of the aerobic biomass is 10 $\mu\ell$ O_2/ hr / mg MLVS. This rate is typical of the endogenous respiration of bacteria.

The residence time of sewage in the treatment units influences both the types of nitrogen compounds present (NH_4^+, NO_2^-, NO_3^-) at any given time, and the rate of nitrogen oxidation. With a well developed biomass, the Activated Bed units converted up to 95% of the incoming NH_3-N, leaving NO_3^--N as the only oxidized nitrogen compound present, in 71 minutes at a rate of 5.2 mg/hr / g MLVS.

The nitrification rate increases with decreasing residence time (increased substrate flow). Efficiency of nitrogen oxidation remains high until substrate excess is achieved.

The Activated bed units contained a MLVS that averaged 5500 mg/ℓ for Unit 1 and 7100 mg/ℓ for Unit 2 or 6300 mg/ℓ for the combined data from the two units.

A mathematical model was developed that accurately predicted the performance of the system during coupled continuous operation and cycling between aerobic and anaerobic modes. Corrections for temperature and ammonia release were also included in the model.

Denitrification rates respond to changes in temperature in a manner typical of biological systems - a doubling in rate for a 10°C rise in temperature. During continuous operation the rates (R) averaged 0.30 mg N removed/hr/g MLVS at 18°C and 0.68 mg N removed/hr/g MLVS at 29°C.

The amount of dissolved oxygen entering with the nitrified feed appears to have no effect on the course of denitrification at residence times of over 400 minutes. This is true even when the incoming feed is oxygen saturated.

The bacterial cells making up the biomass do not appear to undergo major physiological damage with anaerobic periods as long as 70 hours. This is evidenced by the absence of large protein molecules and a relatively low level of amino acids in the recirculating liquor. This indicates that massive cell lysis has not occurred and the cells are not losing their amino acid pools as would be expected under typical anaerobic conditions. Some small nitrogen containing molecules probably originating in the amino acid pools are released into the medium from the cells as evidenced by the low level of ammonia present in the circulating liquor. The rate of ammonia release is 0.15 mg/hr/g. MLVS.

The anaerobic mode appears to be a physiologically "aerobic" environment. This is evidenced by the biochemical data just discussed, the oxidation of some of the residual COD entering the unit, and the rapid recovery of oxidative metabolism when returned to the aerobic mode.

The energy derived from the oxidation of the residual COD entering the denitrification unit is sufficient to account for only 12% of the NO_3-N reduced. Thus the bacteria must be utilizing stored reserves as the principal energy source for the denitrification process.

The stored carbon reserves appear to be capable of maintaining denitrification for up to 70 hours. Extended periods of anaerobiosis up to 165 hours indicate that part of the biomass may be lost, as evidenced by the length of time required for nitrification to be restored.

The overall nitrogen removal efficiency under the test conditions of the program was 70 - 90% of the total Kjeldahl nitrogen contained in the primary effluent feed.

II. RECOMMENDATIONS

As a consequence of the above observations and the fact that the feasibility of the concept was demonstrated using a municipal wastewater on a continuous feed basis, several recommendations for future work can be made. One is that the treatment units should be re-engineered to take into account practical treatment plant operations. Hydraulic loadings should be balanced and pumping and aeration costs minimized.

Preliminary cost estimates based on the present program should be made for a properly engineered system. It would be more appropriate to design the prototype system on some intermediate scale and obtain actual operating data for both costing and plant design purposes. As a consequence it is recommended that the prototype system should have a treatment capacity of 25,000-50,000 gallons wastewater per day.

The recommended program would consist of both the plant design and operation, and a laboratory phase. The bulk of the laboratory effort would be concentrated in the first part of the program. It would be geared to providing inputs for the plant design such as methods of aeration and balancing of hydraulic flows. The mathematical model would also be modified to fit the enlarged and revised system. Other experimental parameters would be checked to act as guidelines for setting up an operational program for the plant.

The pilot plant phase should be designed to obtain actual operating data for engineering purposes and accurate cost estimates. The overall efficiency of the process should be evaluated and cost vs. removal efficiency estimates obtained. Operational modes should also be studied to simplify control of the overall operation. The information generated in this recommended program could then be used to design a full-scale demonstration plant.

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III. INTRODUCTION

Numerous methods for accomplishing the removal from wastewaters of the nutrients, nitrogen and phosphorus have been proposed. Chemical processes for both nitrogen and phosphorus reductions are currently under investigation at various installations. The control of phosphate will probably continue to be dependent on a chemical system, but nitrogen removal can be accomplished biologically and when properly exploited has the potential of being both efficient and economical.

Nitrogen can be removed from a wastewater by two biological processes. As ammonia it can be assimilated by living cells which incorporate it into proteinaceous cell material, or as nitrate it can be the substrate for denitrification, the biological conversion of inorganic nitrate to free N_2 or N_2O gas. For the former process to be an effective mode of removal the nitrogen contained in the wastewater would have to be the limiting nutrient to assure that it is all incorporated into the cell material. All cellular organic material must then be separated from the waste effluent, and the generated cellular waste must be disposed of in a manner such that it does not again become a source of pollution. The second method is potentially more effective, since nitrates are volatilized to nitrogen gas, which then escapes into the atmosphere.

In the course of biological conversion of nitrate ion to nitrogen gas, carbon compounds are oxidized. Herein lies a current problem in utilizing denitrification to remove nitrogen from wastewaters on a large scale. In a typical wastewater treatment effluent which is amenable to denitrification by virtue of the oxidized state of its inorganic nitrogen, dissolved organic carbon has also been so oxidized that very little remains to serve as a source of energy for the microorganisms. Hence, in most biological denitrification processes under investigation (McCarty, et al, 1969), an external source of organic carbon must be supplied. Methanol is the favored compound.

The program described in this report explored the use of the stored intracellular food reserves of the aerobic microflora as the source of oxidizable substrate. The principal goal of the study was to define the conditions under which an aerobic, oxidizing microflora could be made to denitrify, without the addition of supplementary carbon. An additional aspect of this program is that all of the information generated is basic to biological processes therefore applicable to other denitrification systems.

NITRIFICATION

True biological denitrification, or non-assimilative removal of nitrogen, is performed only on the oxidized forms of nitrogen. The oxidation of ammonia, or biological nitrification, is thus essential to nitrogen removal. To assure complete denitrification, nitrification must also be complete.

Nitrification is performed by chemoautotrophic bacteria, which fix CO_2 as a source of carbon for cell material and obtain energy for the process by oxidizing inorganic substrates. Two groups of the chemoautotrophs are distinguished, each responsible for a specific phase of the nitrification process. The first group, consisting of the genera Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocystis, and Nitrosogloea, can oxidize ammonia to nitrite. The second group, comprised of the genera Nitrobacter and Nitrocystis, is capable of oxidizing nitrite to nitrate. Total oxidation of the nitrogen in wastewater to nitrate is therefore a two-step process, each step being performed by different microflora. Simple chemical descriptions of the two phases are:

$$(1) \quad NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$$

$$(2) \quad NO_2^- + 0.5O_2 \longrightarrow NO_3^-$$

The first phase of this program was directed towards an examination of the behavior of the microflora during the aerobic oxidation of wastewater. Principal emphasis was placed on defining the conditions that control nitrification and on attaining complete oxidation of the inorganic nitrogen in wastewater, as a preface to subsequent studies of denitrification.

DENITRIFICATION

Biological denitrification is technically an anaerobic process wherein the nitrate ion fulfills the role normally occupied by oxygen in aerobic respiration--that of hydrogen ion acceptor in the electron transport system. In other words, in the bacterial species which are capable of denitrifying, the presence of nitrate ion permits the microbial cell to maintain "aerobic" metabolism in the absence of oxygen. In the process, nitrates are reduced to nitrogen gas and carbon compounds are oxidized. This reaction is represented by the following equation:

$$4NO_3^- + 4H^+ + 5CH_2O \longrightarrow 5CO_2 + 2N_2 + 7H_2O$$

Most of the denitrifying organisms are aerobes and are commonly found in the wastewater treatment plant. These include species of Pseudomonas, Achromobacter, Bacillus, and Micrococcus.

Since the growth of these microorganisms is not dependent on the reduction of nitrate, the presence of a large denitrifying population does not in itself indicate that conditions are suitable for denitrification. The existence of a large population, on the other hand, does point to a significant denitrifying potential. It was the objective of the second phase of this program to explore this potential and to define the conditions necessary for maximum nitrogen removal.

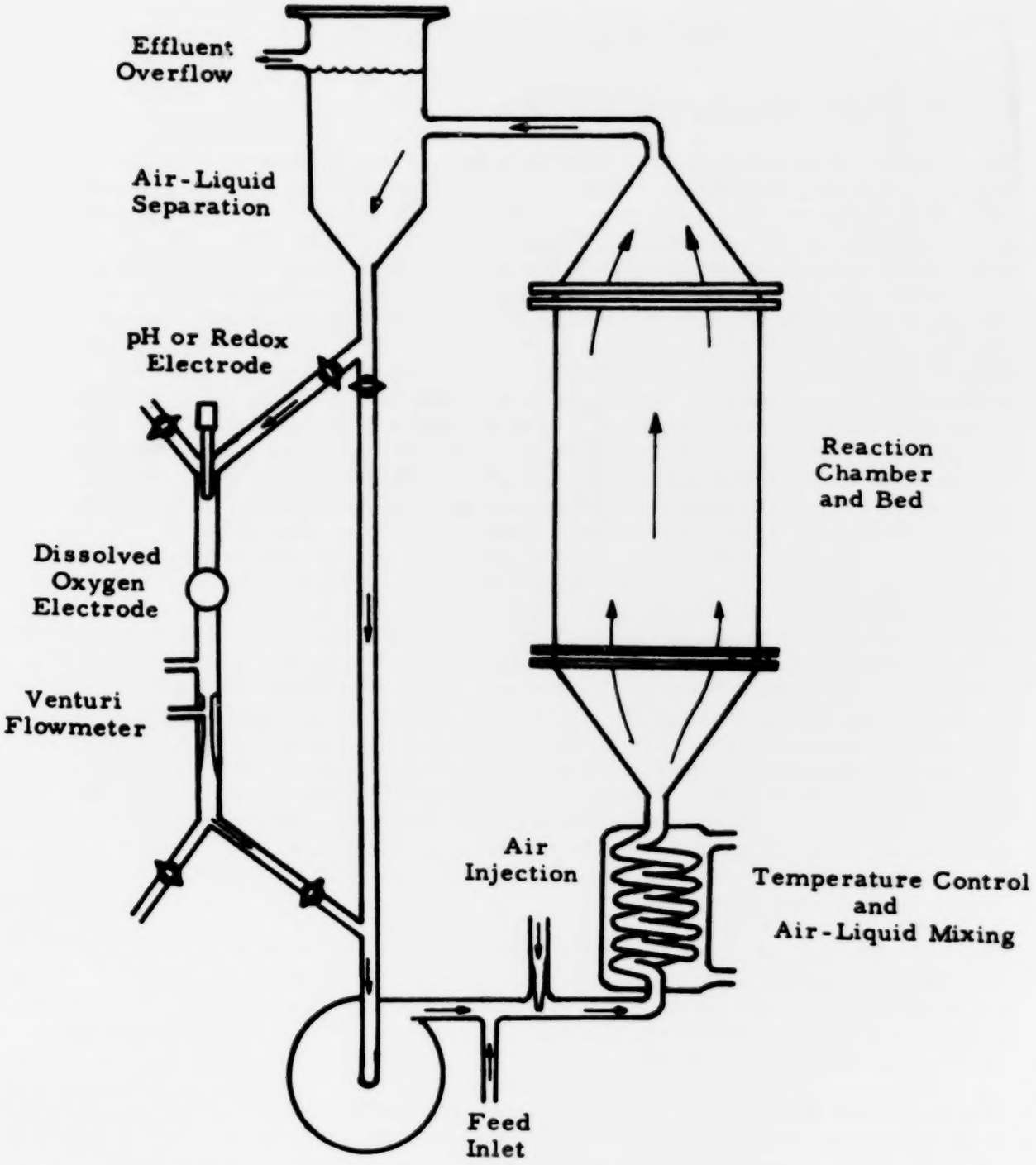
IV. MATERIALS AND METHODS

LABORATORY TREATMENT DEVICES

The sewage treatment system used throughout the program incorporated flooded reaction chambers, inside of which the wastewater being treated is continuously recirculated over a dense mass of microorganisms fixed to the surface of plastic rings. Figure 1 diagrammatically shows a laboratory scale treatment device employing this design. For aerobic operation, air is injected at the discharge side of the recirculation pump into the circulating liquid which then passes through a jacketed coil to provide thorough mixing. Water from a controlled-temperature water bath is circulated through the mixing coil jacket to maintain precise operational temperatures for the entire activated bed unit. Wastewater to be treated is metered into the recycle, and the treated effluent overflows at the same rate along with excess air at the separation chamber. The experimental units employ an instrumentation shunt which allows simultaneous measurement of recirculation flow, dissolved oxygen content of the liquid leaving the reaction chamber, and either pH or oxidation-reduction potential, without disturbing the operation of the unit. Recirculation flow is monitored by using a glass venturi flowmeter which measures pressure drop as a function of change in velocity of the liquid as it passes through the venturi. The other measurements are accomplished with electrodes and probes. Under normal operating conditions, the liquid flows directly from the separation chamber to the recirculation pump; the liquid is bypassed through the instrumented shunt for measurement. The shunt allows easy maintenance of the probes without shutdown of the unit, and allows unimpeded recirculation flow under normal operation. Slime buildup due to biological growths in the venturi flowmeter, which would change its hydraulic characteristics, is also minimized. Figure 2 shows the two mirror-image activated bed units, using a common measuring shunt with associated instrumentation, as installed in our laboratory.

The bed chamber of each activated bed unit is filled with packing made of 3/4-inch lengths of 3/4-inch O. D., 1/2-inch I. D. PVC tubing. Pertinent dimensions of the units are presented in Table 1. The volumes quoted in the table represent the system under normal operation and do not include the instrumented shunt.

The activated bed units as described are assembled for reasons of laboratory convenience, and are not intended for strict scale-up to plant size. By their design they allow precise control of experimental conditions which is essential if reproducible results are to be obtained.



ACTIVATED BED UNIT

Figure 1

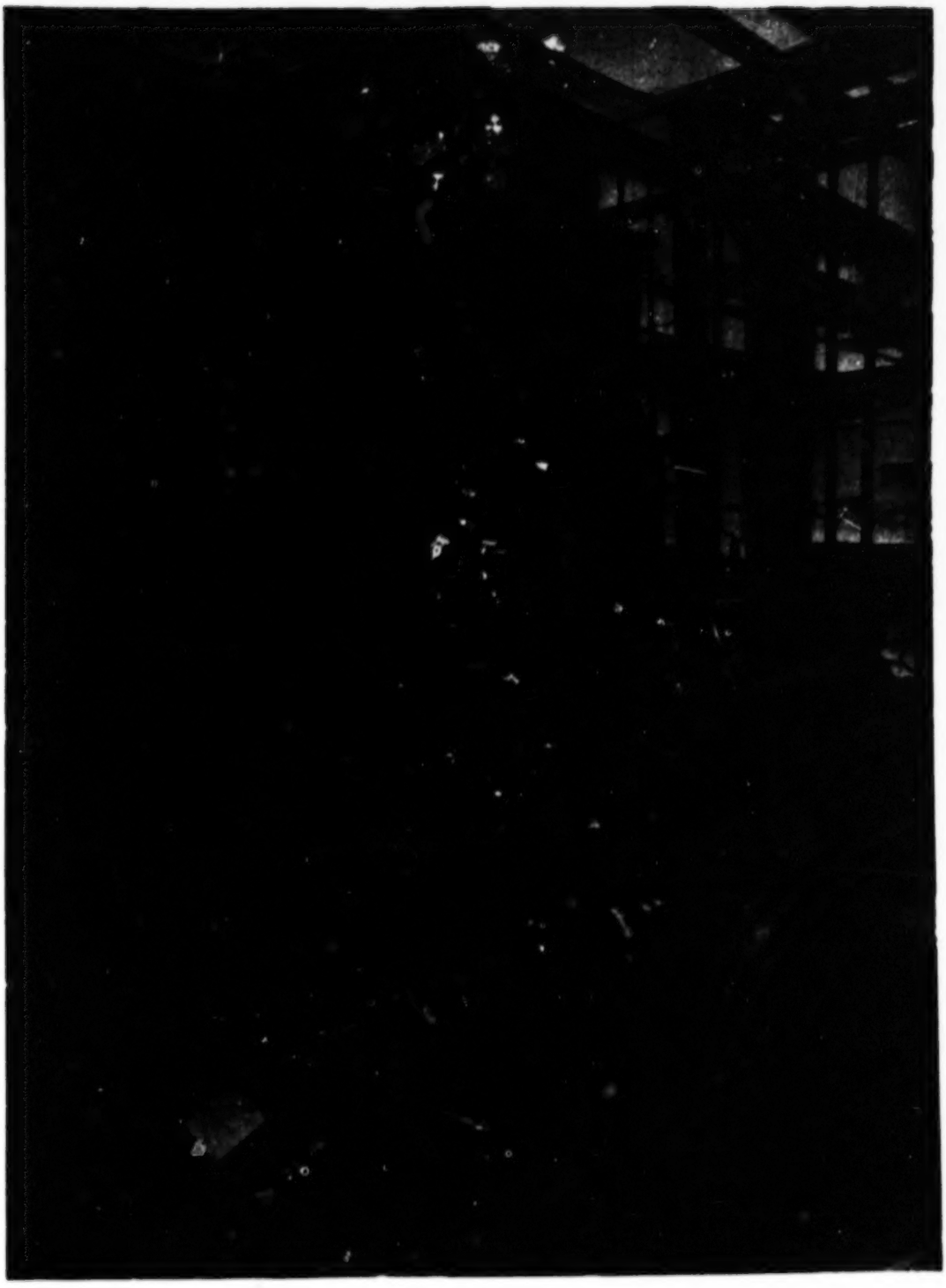


Figure 2. The Two Mirror Image Activated Bed Units Used in the Experimental Program. The interconnecting instrumentation by-pass is visible between the units.

TABLE 1

DIMENSIONS OF ACTIVATED BED UNIT

Total system volume, with packing, but without bed growth	3740 ml
Total system volume, with packing, and bed growth (air off)	2710 ml*
" " " " " " " " (air on)	2400 ml*
Volume of bed growth and trapped "noncirculating" liquid	1030 ml
Volume of bed chamber with packing, without bed growth	2500 ml
Percent of system represented by bed chamber, by volume	67.0 %
Surface area of bed chamber and packing, without growth	8500 cm^2
Surface area of separation chamber, air mixing chamber, and associated tubing, without growth	1830 cm^2
Total surface area, without growth	10330 cm^2
Percent of system represented by bed chamber, by surface area	82.2 %
Average thickness of growth, total system ($1030\ cm^3/10330\ cm^2$)	1.0 mm

*Average system volume used for calculations in this report = 2500 ml.

ANALYTICAL TECHNIQUES

The various analytical procedures used in gathering the data contained in this report are described in Appendix I.

WASTEWATER FEED FOR THE STUDIES

The sewage used throughout this program was primary effluent obtained from the Azusa, California, municipal sewage treatment plant. It was procured three times weekly and stored in a 500-gallon, trailer-mounted aluminum tank. A recirculation pump was installed in the tank to keep the system from going anaerobic and the solids suspended. Pertinent average analytical data is presented in Table 2. These analyses were carried out on each tank of sewage when the actual data gathering experiments were in progress, and represent about three analyses per month. Composition of the sewage remained relatively constant throughout the course of the year's study, as indicated by the standard deviations in Table 2. This enabled valid comparison of data obtained early in the year with that obtained later in the course of the work.

TABLE 2

CHARACTERISTICS OF AZUSA PRIMARY EFFLUENT
(Average of 35 Samples) +

Characteristic	Average Value	Standard Deviation
Chemical Oxygen Demand (COD)	188 mg/l	± 18 mg/l
Total Kjeldahl Nitrogen (TKN)	49.3 mg/l	± 4.6 mg/l
Ammonia Nitrogen (NH_3-N)	40.1 mg/l	± 4.2 mg/l
Organic Nitrogen *	9.2 mg/l	-
Nitrate Nitrogen (NO_3-N)	0.05 mg/l	± 0.05 mg/l
Nitrite Nitrogen (NO_2-N)	0.05 mg/l	-
pH	7.84	± 0.09
Redox Potential	-246 mv	± 35 mv

+ Approximately three analyses per month.

* Obtained by subtracting NH_3-N from TKN.

V. AEROBIC OXIDATION IN THE ACTIVATED BED UNITS

RATIONALE OF THE EXPERIMENTAL PROGRAM

The activated bed unit is essentially a continuous flow microbial culture device in which the growth rate of the microflora can be controlled. As in other wastewater treatment processes, the ultimate objective of the activated bed unit in continuous aerobic operation is to effect maximum oxidation of incoming wastes. Ideally, carbon compounds are oxidized to carbon dioxide and nitrogenous compounds are oxidized to inorganic nitrate.

Carbon oxidation and nitrogen oxidation, however, are performed by two different types of bacteria. Heterotrophs represented by Zooglaeae, Pseudomonas, and Chromobacter require preformed organic carbon as a food and energy source. The chemotrophs of which the nitrifying groups of Nitrosomonas, and Nitrobacter are representative are able to fix CO_2 as their carbon source utilizing the oxidation of inorganic ions as a source of energy. The fact that the two principal biological groups involved in aerobic waste treatment have such divergent, nutritional needs, leads to complications in attempting to simultaneously oxidize both the carbon and the nitrogen.

As a generalization, the chemotrophs grow at a much slower rate than do the heterotrophs. In a continuous culture system involving a mixed microbial flora and a fixed feed rate, differences in growth rate have a profound effect on the proportion of the total population a given group may represent.

Thus, when a treatment system is operated at short residence (detention) times, the heterotrophs predominate in the biomass. As residence times are increased the slower growing organisms are able to remain in the system and due to substrate limitation the growth rate of the heterotrophs is slowed down. With the increased availability of substrate, i.e., ammonia, there occurs a change in the relative growth rates of the two groups and the proportion of nitrifiers increases. The overall effect of lengthening residence times is to increase the sludge age. Increasing the sludge age insures both oxidation of all of the incoming carbon and complete nitrification of the incoming nitrogen.

The principles discussed above were tested in the activated bed units. This was necessary to permit poising the system at the desired levels of oxidation to provide a nitrified feed to the anaerobic system. The stability of the biomass was an additional consideration since complete recovery was desired after the anaerobic phase.

EFFECTS OF ORGANIC LOAD AND RATE OF OXIDATION

Aerobic experimentation with the treatment units centered around defining the relationship between the average length of time an increment of sewage feed spends in contact with the microflora and the degree of carbon or nitrogen oxidation achieved. Residence time is computed as $\overline{t} = \frac{v}{f}$, where v is the free liquid volume of the unit and f is the input flow rate. Degree of carbon and nitrogen oxidation was also examined as a function of organic loading, which is inversely proportional to residence time for a sewage feed containing a given concentration of organics. Organic loading was calculated as

$$\text{Organic load} = \frac{f(\ell/\text{min}) \times \text{COD } (g/\ell) \times 1440 \text{ min/day}}{\text{MLVS } (g/\ell) \times L}$$

where MLVS is the total volatile solids of the entire treatment unit (MLVS data will be presented in a later section).

Figure 3 shows the relationship between organic load and rate of COD removal in both of the treatment units. The data were obtained over a three-month period by operation at various feed flows. Temperature was maintained at 29° ± 1°C, and recirculation rate within the units was held constant at 2 lpm. Air flow into the units was 2.1 slpm each. At this air input and recirculation rate, the dissolved oxygen concentration in the liquor leaving the reaction chamber of each unit was 7.5 mg/ℓ. Since the solubility of oxygen in air-saturated water at 29° at sea level is 7.7 mg/ℓ, the liquid leaving the reaction chamber was essentially saturated. The results plotted in Figure 3 show a rapid increase in rate of COD oxidation as organic loading is increased, to the point (at an organic load of 1.1 g COD/g MLVS/day) where COD oxidation rate reaches a limit.

The relationship between metabolic rate and organic loading is a type of saturation curve, as described by Herbert (1961) for the bacterial chemostat. Growth rate is proportional to substrate concentration when this is low but approaches a maximum value as the substrate concentration increases. At maximum growth rate, substrate utilization should also be maximal and supplying substrate over and above this amount would mean that only a portion would be oxidized and overall efficiency would drop.

Figure 4 presents the overall nitrification results obtained in these first aerobic runs as percent oxidation of total Kjeldahl nitrogen (TKN). TKN values are the sum of ammonia plus organically bound nitrogen, and do not include inorganic nitrite and nitrate. In the instances of high oxidation of TKN, all the ammonia and a small fraction of the organically bound nitrogen were found to have been oxidized to nitrite and nitrate (see Figures 6 and 7). Figure 4 shows that nitrification was complete for organic loads up to 1.1 g COD/g MLVS/day. At loadings greater than that level, degree of nitrification drops as a function of the increased loading.

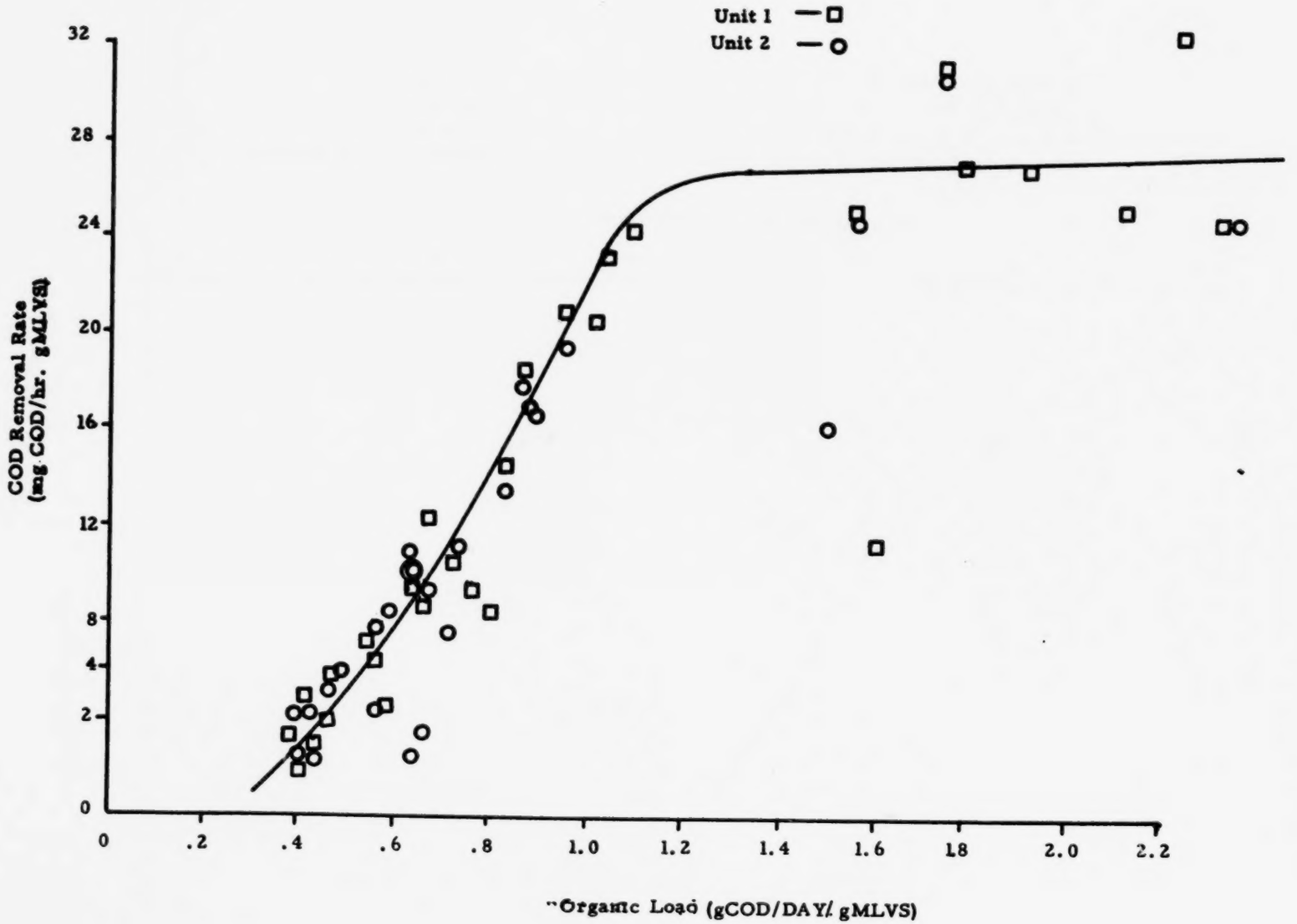


Figure 3. Effect of Organic Load on COD Removal Rates - Aerobic Mode. Units were operated over various flow rates over a three-month period at 29°C. Unit 1 = 5.5 g MLVS; Unit 2 = 7.1 g MLVS (Table 4).

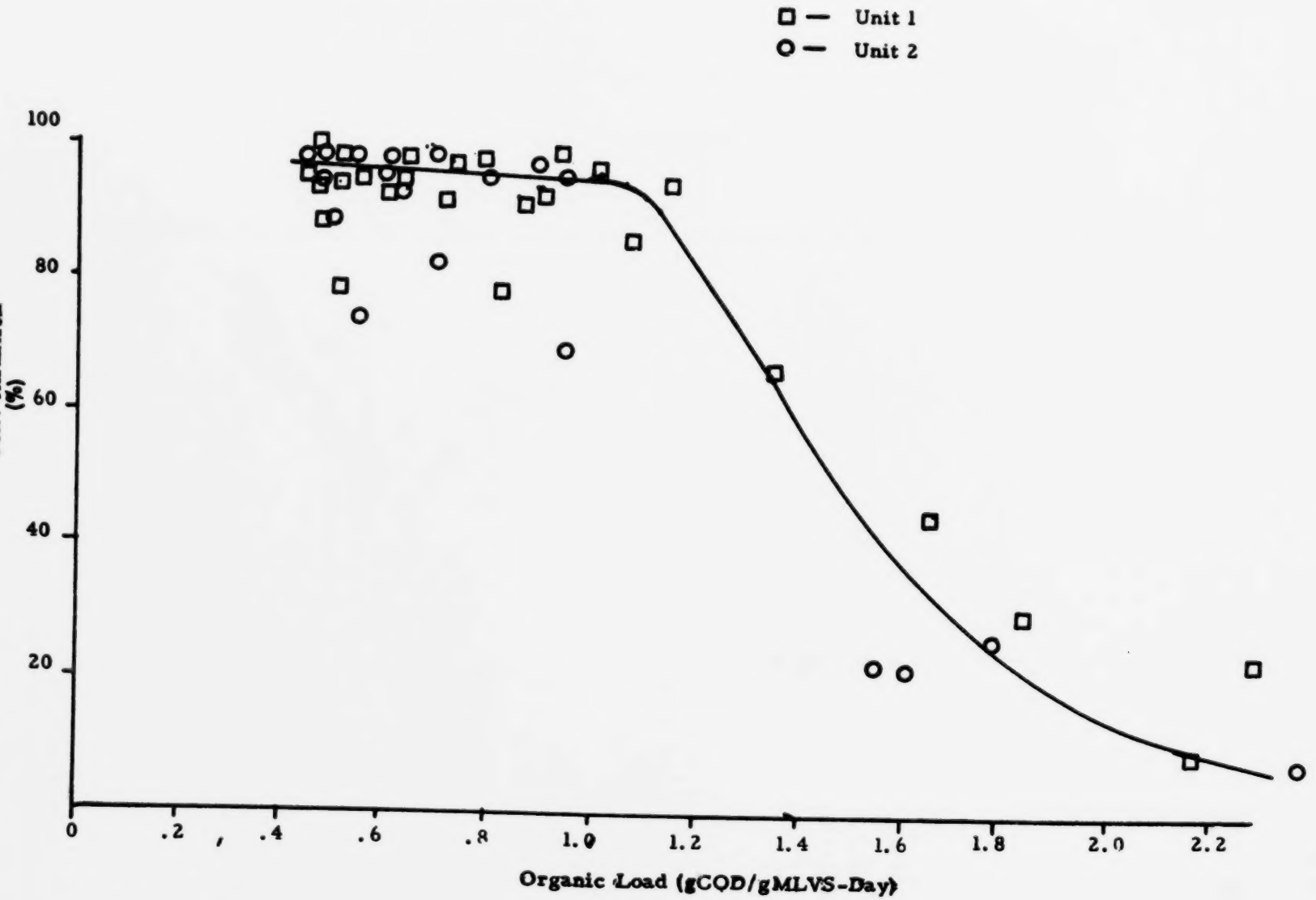


Figure 4. Oxidation of Total Kjeldahl Nitrogen (TKN) as a Function of Organic Load. Aerobic mode - 29° C. Average incoming nitrogen in Azusa feed - 49.3 mg/l (Table 2); Unit 1 = 5.5 g MLVS; Unit 2 = 7.1 g MLVS (Table 4).

UTILIZATION OF DISSOLVED OXYGEN IN INCOMING FEED

The rate of dissolved oxygen utilization in an actively nitrifying aerobic unit was determined to establish the optimal oxygen requirement of the microflora. The procedure used was to monitor the oxygen content of the liquid flowing past an oxygen sensor placed in the circulating liquor. At zero time the air flow to the unit was turned off and changes in the oxygen level followed with respect to time. To check the base lines the air was turned back on until the concentration returned to the original starting (zero time) level. Various modes of operation were tested but the one of significance was to maintain all conditions constant including feed and recirculation and merely turn off the air. Feed rate was set at 20 ml/min and temperature was 29°C.

A typical series is shown in Figure 5. The shape of the oxygen consumption curve is highly reproducible and at least for this series, is independent of the starting concentration at time zero. Because of difficulties in calibrating the instrument while in the system, values below 0.5 mg/ℓ O_2 cannot be considered as significant. By employing the initial maximum slope of this oxygen utilization curve, a rate of oxygen uptake by the microflora can be obtained. In unit 2, 1.5 mg O_2/liter/min is used. Converted to $\mu\ell$ O_2/liter/hr:

$$\frac{1500\ \mu g\ O_2}{\text{liter/min}} \times \frac{60\ \text{min}}{\text{hr}} \times \frac{22.4\ \mu\ell\ O_2}{32\ \mu g\ O_2} = \frac{63,000\ \mu\ell\ O_2}{\text{liter/hour}}$$

The oxygen utilization quotient (Q_{O_2}) can be obtained by dividing this number by the biomass concentration of the bed:

$$Q_{O_2} = \frac{\dfrac{63,000\ \mu\ell\ O_2}{\ell/\text{hr}}}{7100\ \text{mg MLVS}/\ell} = \frac{8.9\ \mu\ell\ O_2}{\text{mg MLVS/hr}}$$

These Q_{O_2} values can be compared with those in published bacteriological literature. The Q_{O_2} for growing bacterial cultures is strongly influenced by the age of the cells and the substrate being metabolized. Cultures of E. coli when actively growing have been reported to have a Q_{O_2} 25 - 75 $\mu\ell$ O_2/hr/mg dry bacterial weight (Spector, 1956). The Q_{O_2} for the biomass of the activated bed units is low when compared to cells metabolizing in the logarithmic phase. Dawes (1963) reports values for the endogenous Q_{O_2} of E. coli of from 15.5 - 29.3 $\mu\ell$/hr/mg dry weight. If one considers that the volatile solids of the activated bed biomass averages 72% Unit 1; 87% Unit 2 (Table 4) and converts to a total dry weight basis, the activated bed Q_{O_2} ranges from 12.5 - 16.2 $\mu\ell$ O_2/hr/mg dry weight. A Q_{O_2} of 12.5 is more typical of an endogenous bacterial respiration and reflects the fact that the unit is poised at the end of the log phase in the bacterial growth curve wherein the metabolism of the organisms has started to slow down.

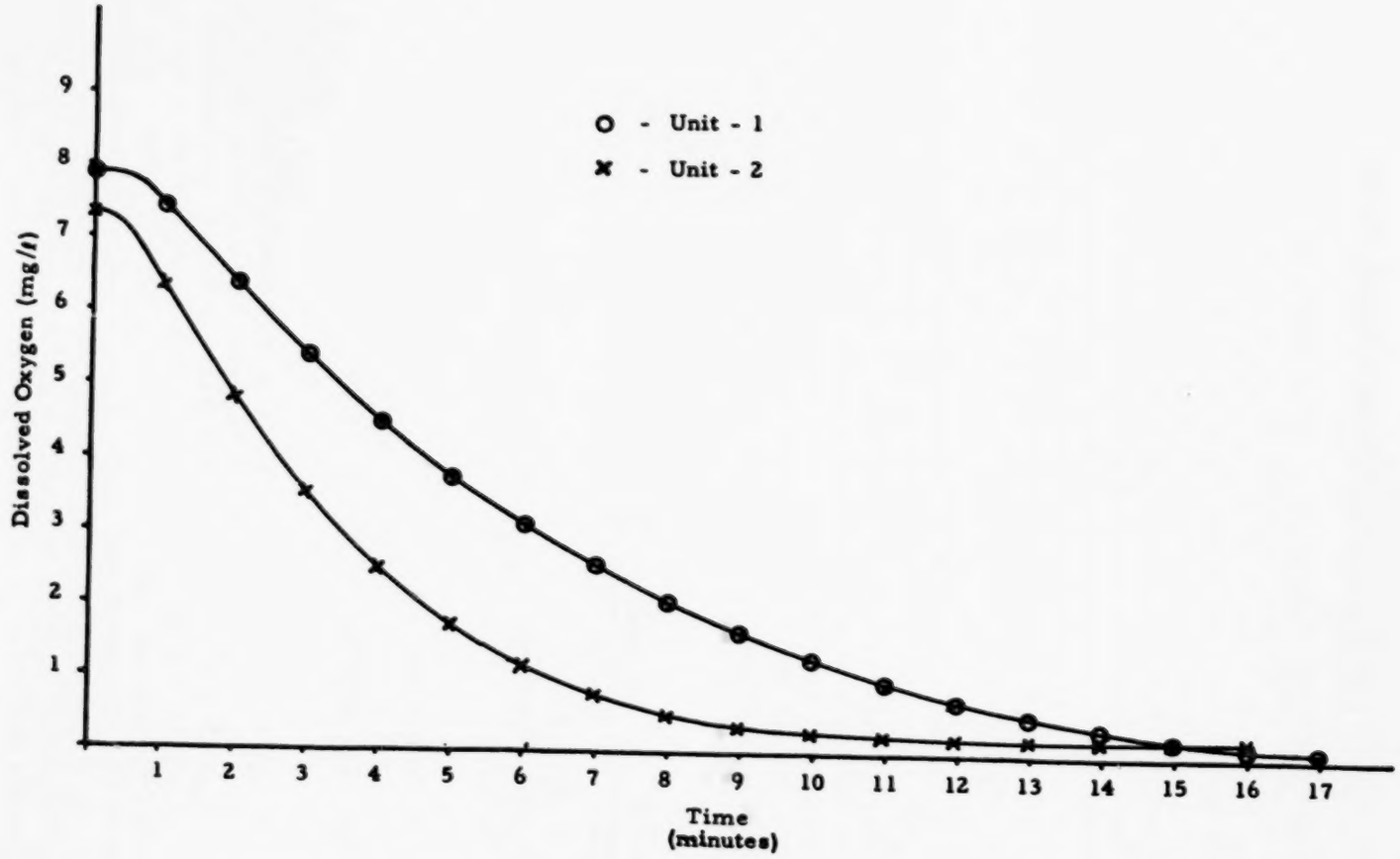


Figure 5. Dissolved Oxygen Utilization by the Microflora. Air Feed into the units turned off at time = 0. Flow rate of Azusa feed was maintained at 20 ml/min and temperature at 29° C. Unit 1 = 5.5 g MLVS; Unit 2 = 7.1 g MLVS.

NITRIFICATION AS A FUNCTION OF RESIDENCE TIME

A study of the relationship between degree of nitrification and influent flow for primary effluent revealed that the residence time of sewage in the treatment unit is the controlling parameter. Not only does residence time control the amount of ammonia oxidized but also the quantitative mixture of the three principal nitrogen species, NH_4^+, NO_2^- and NO_3^-.

Figures 6 and 7 derived early in the program show the expected two-phase pattern of ammonia to nitrite and then nitrite to nitrate conversions. Differences in the two treatment units reflect different histories such as time at various feed rates, temperatures, etc. At input flows that give residence times of from 30-120 minutes, significant levels of nitrite appear. Only after about 120 minutes is all of the ammonia-nitrogen present as nitrate. A simple rate calculation can be made for the overall conversion of NH_4 to NO_3.

Given: NH_4-N converted to NO_3-N = 40 mg/ℓ (from Table 2)
Residence time = 2 hrs (5% NH_4 remaining, Figures 6 and 7)
MLVS = 6.32 g/ℓ (Table 4)

Therefore, the rate of conversion of ammonia to nitrate =

$$\frac{40 \text{ mg } NO_3\text{-N/L}}{2 \text{ hrs } 6.32 \text{ g/L MLVS}} = \frac{3.2 \text{ mg } NO_3\text{-N}}{\text{hr} \cdot \text{g MLVS}}$$

The fact that NO_2^- accumulates as an intermediate indicates that under these test conditions the NO_2^- to NO_3^- conversion is rate limiting for residence times up to 60 minutes.

After two months of continuous operation, a similar set of experiments was carried out monitoring the appearance of the oxidized nitrogen species as a function of residence time. In this case only transient amounts of nitrite were detected, as shown in Figure 8. The overall rate of conversion of $NH_4 \longrightarrow NO_3$ also improved.

$$R_{(NH_4 \text{ to } NO_3)} = \frac{40 \text{ mg/L } NO_3\text{-N}}{1.22 \text{ hrs} \cdot 6.32 \text{ g/MLVS}} = \frac{5.2 \text{ mg } NO_3\text{-N}}{\text{hr} \cdot \text{g MLVS}}$$

The difference in the two rates of oxidation indicates a shift in the proportion of the metabolic activity represented by the NO_2^- oxidizing organisms. Every indication is that the NH_4^+ to nitrite conversion is now the rate limiting step and that as soon as a molecule of nitrite is formed it is immediately converted to the nitrate.

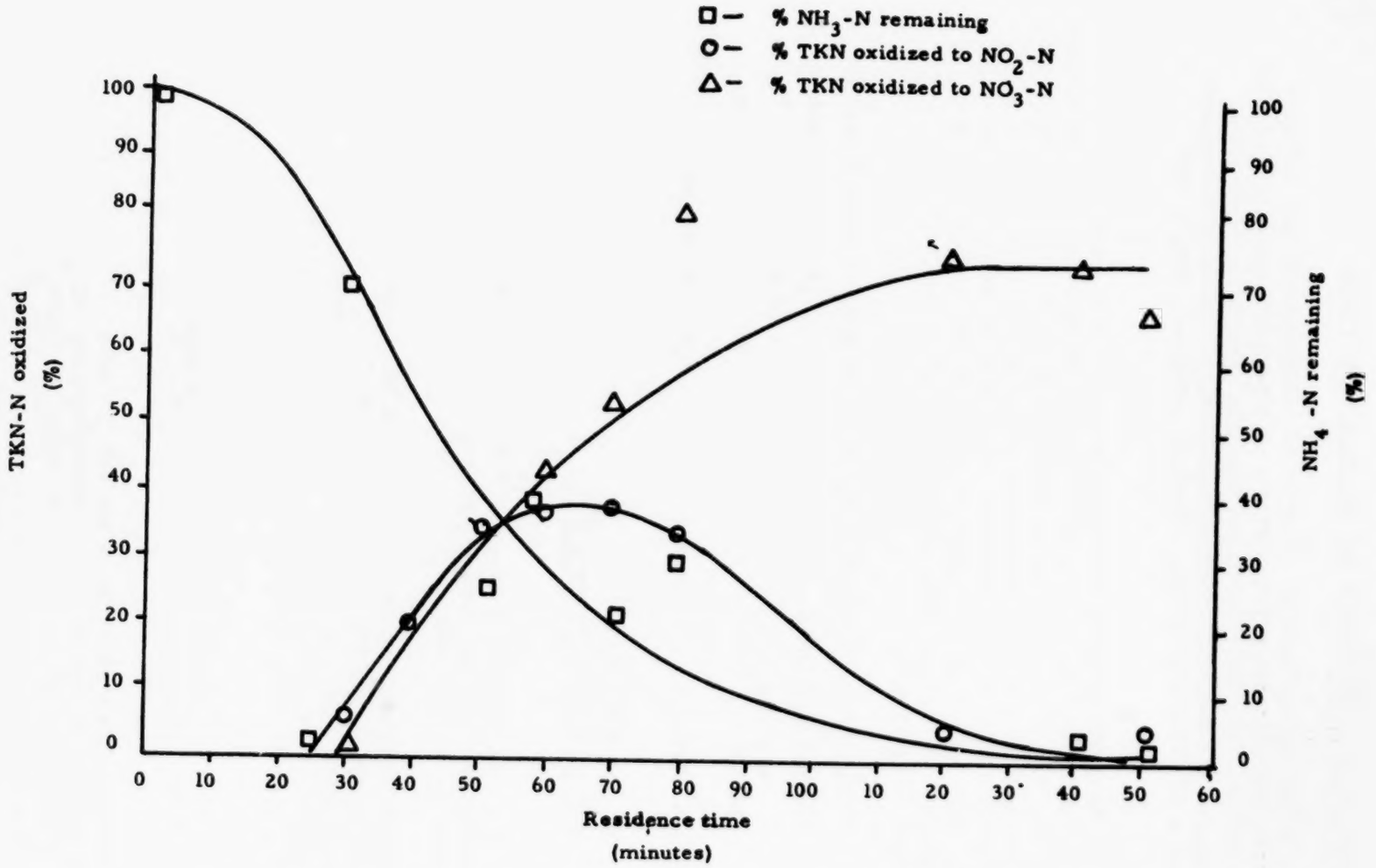

□— % NH_3-N remaining
○— % TKN oxidized to NO_2-N
△— % TKN oxidized to NO_3-N
TKN-N oxidized (%)
NH_4-N remaining (%)
Residence time (minutes)
0 10 20 30 40 50 60 70 80 90 100 10 20 30 40 50 60

Figure 6. Nitrogen Transformations in Unit 1 as Effected by Residence Times. Aerobic mode - 29°C. Flow rates of Azusa primary effluent were adjusted to give the indicated residence times. One week of continuous operation at each setting was used to stabilize the unit prior to analyzing the effluents. MLVS = 5.5 g/l.

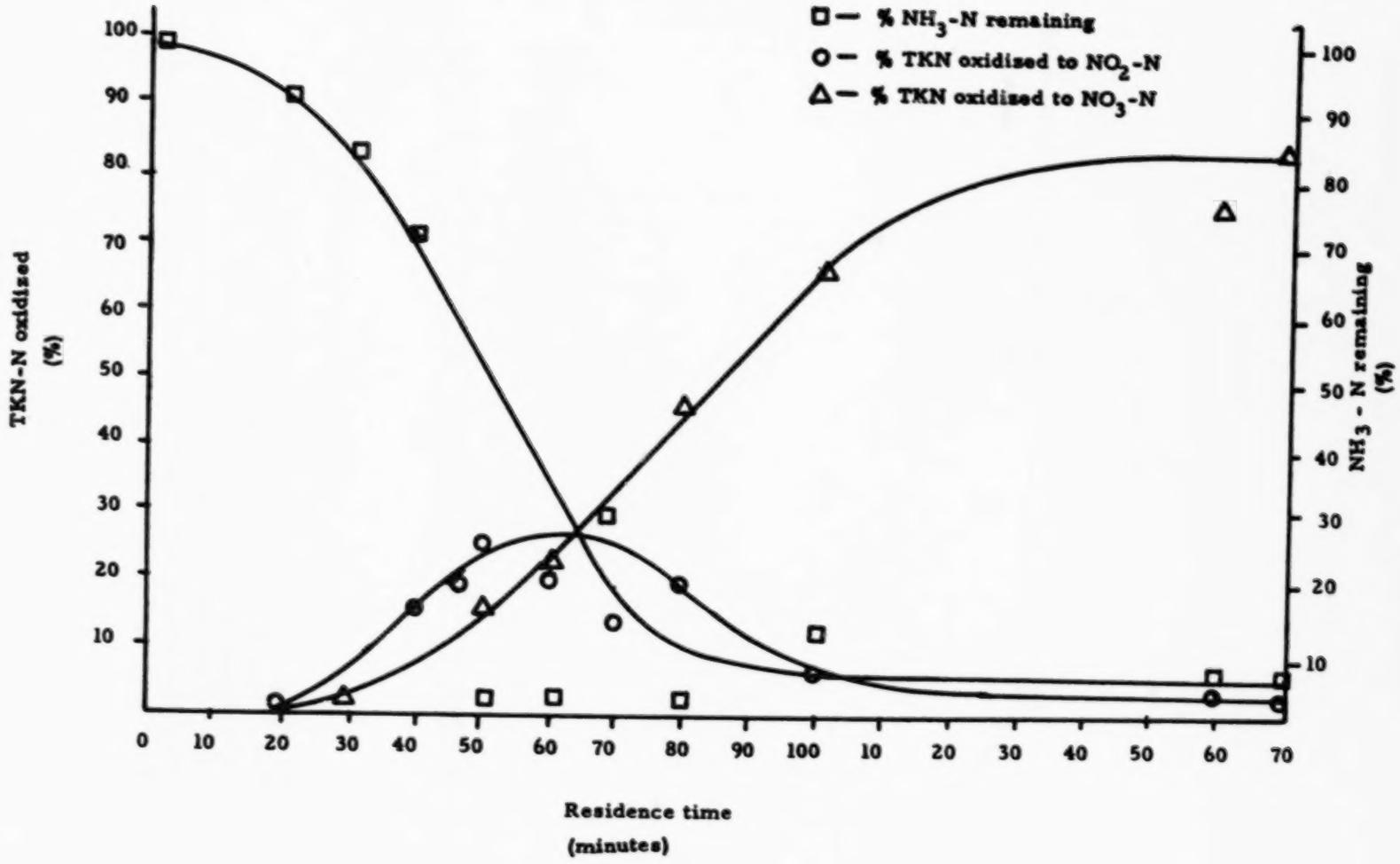

□— % NH_3-N remaining
○— % TKN oxidised to NO_2-N
△— % TKN oxidised to NO_3-N
TKN-N oxidised (%)
NH_3 - N remaining (%)
Residence time (minutes)
0 10 20 30 40 50 60 70 80 90 100 10 20 30 40 50 60 70

Figure 7. Nitrogen Transformations in Unit 2 as Affected by Residence Times. Aerobic mode - 29°C. Flow rates of Azusa primary effluent were adjusted to give the indicated residence times. One week of continuous operation at each setting was used to stabilize the unit prior to analyzing the effluents. MLVS = 7.1 g/ℓ.

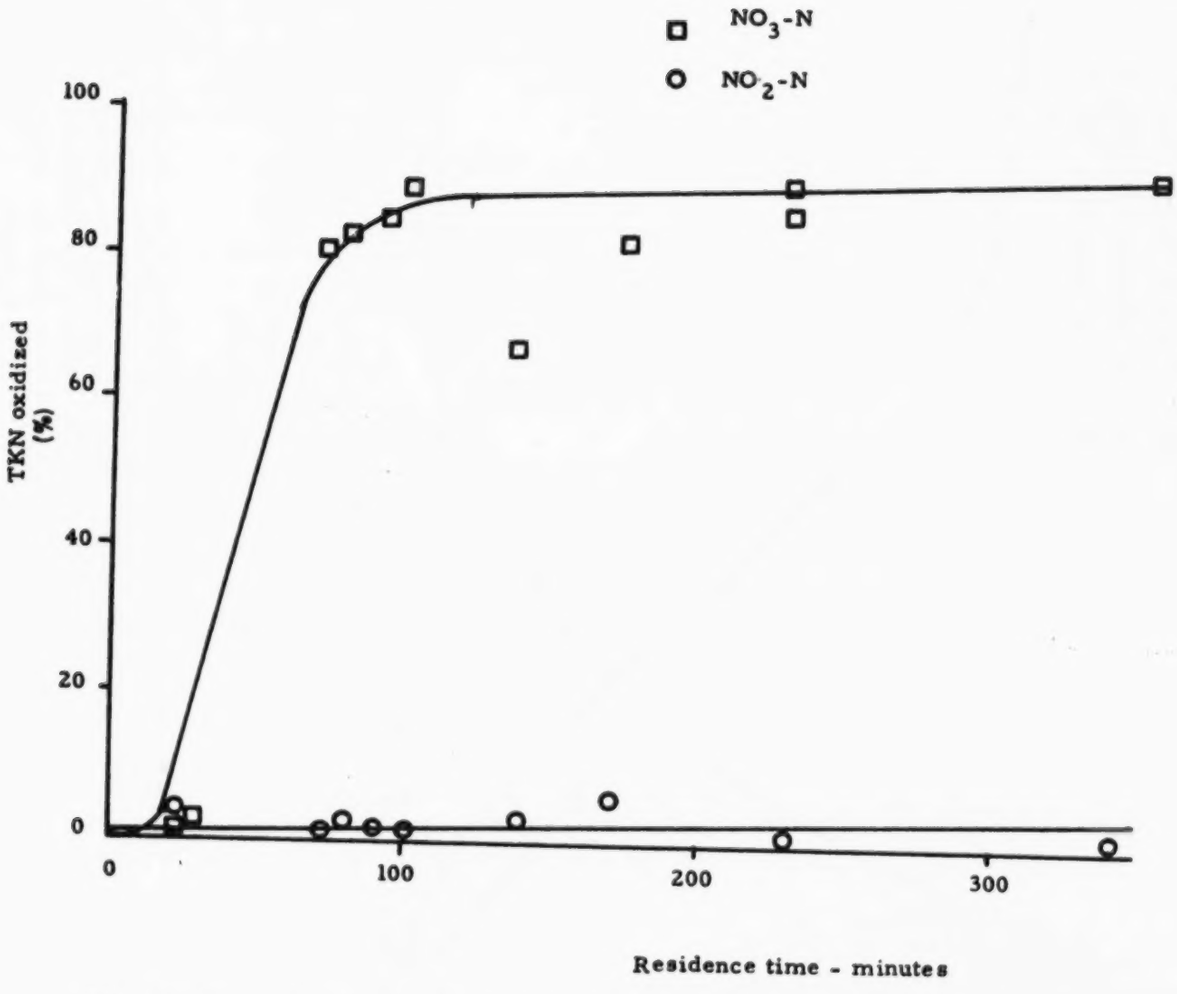


Figure 8. Nitrogen Transformations in the Activated Bed Units After Two Months of Adaptation. System was operated continuously in the aerobic mode at 29°C. Flow rates of incoming sewage were adjusted to give the indicated residence times. Data from Unit 1 and Unit 2 were averaged together.

The data for nitrite production presented in Figures 6 and 7 can be averaged and presented as a function of residence time as in Figure 9.

Comparison of Figures 8 and 9 shows that while there was apparent adaptation of the second stage nitrifiers or those that oxidize nitrite to nitrate, there was no noticeable change in the rate of total ammonia oxidation as shown in the nitrite curves of Figures 6 and 7 in the two months of experimentation. The rate of production of nitrite from ammonia remained stable, indicating early rapid acclimation of the culture to first stage nitrification. In the activated bed system, therefore, nitrification is distinctly a two-stage process, with the microflora responsible for each stage behaving as distinctly separate populations.

RATE OF NITRIFICATION

It is expected that the longer the residence time of the sewage in the activated bed units the larger the proportion of the incoming nitrogen that will be oxidized. However, as residence time is increased the rate of nitrification is found to decrease. Table 3 presents a summary of all of the aerobic runs carried out in the first six months of the program. The rate versus time data is also presented in Figure 10. There is a marked rate change at between 70-100 minutes which roughly correspond with a total oxidation of 85-90% of the incoming nitrogen. In operation of the activated bed units then, there appears to be a trade-off point between a rapid rate and amount of organic nitrogen oxidized.

From these studies of residence time vs nitrate and nitrate in the aerobic units presented in Table 3, it is possible to obtain a lower limit to the rate of nitrification. For the residence time data at 74 minutes for Unit 1 and 71 minutes for Unit 2, a minimum rate of nitrification of 5.2 mg/hr/g MLVS is calculated at 30°C for both units. The actual rate is probably slightly higher, but residence time data was not collected for the period 30-70 minutes, for which times a better estimate of the nitrification rate might have been calculated.

BIOMASS DEVELOPMENT IN THE ACTIVATED BED UNITS

As the critical nitrification and denitrification phases of the program were completed, the units were disassembled at the end of these periods and the biomass was removed from the packing rings of the bed for dry weight and volatile solids determinations. Table 4 gives the results obtained at these times. When the units were reassembled and placed in aerobic operation again, it was observed that the biomass regrew to occupy the clean reaction chambers more rapidly than during the start-up period at the beginning of the program. This was undoubtedly due to the re-seeding effect of small amounts of residual biomass not removed by the cleaning operation. Past experience had shown that the initial start-up period of a new bed is critical. At this stage the microflora is most susceptible to predation by other organisms and changes in its external environment.

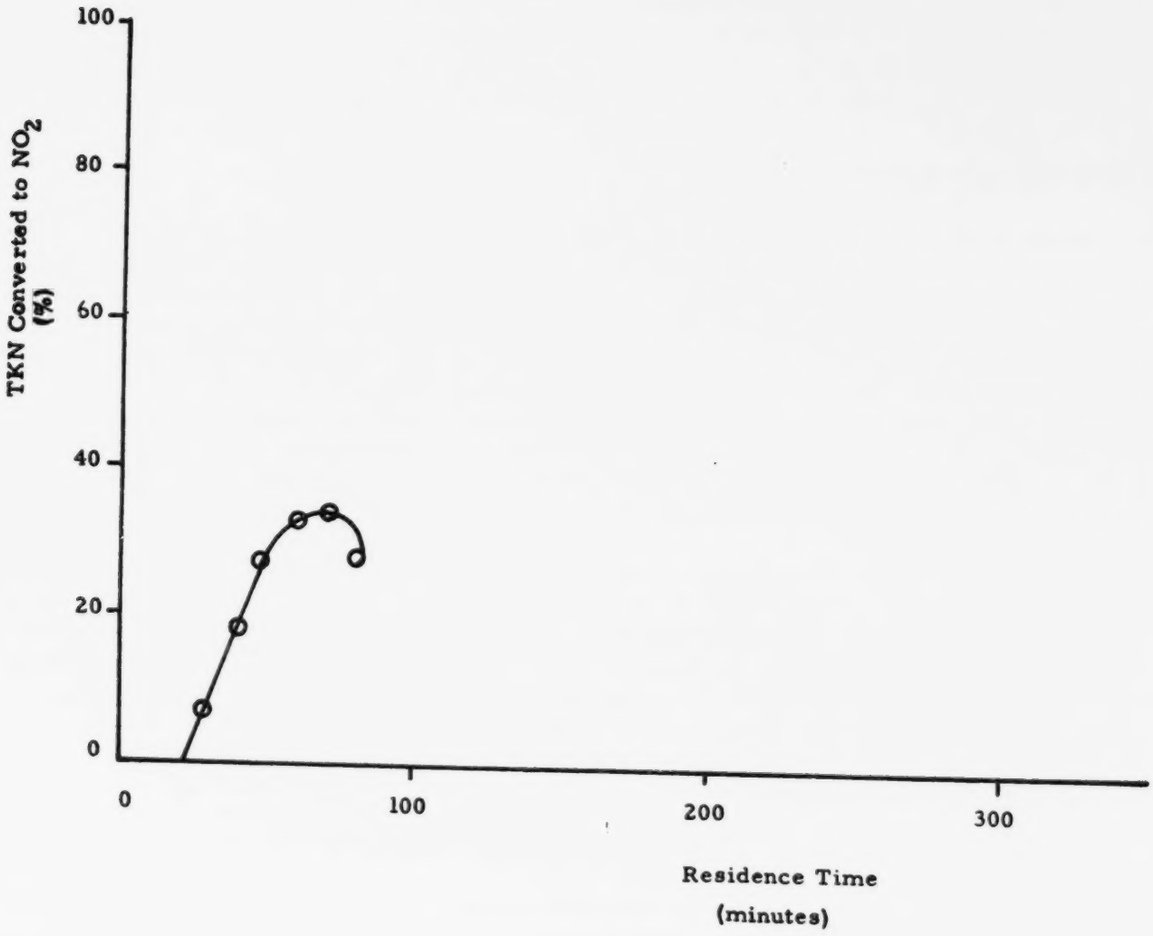


Figure 9. Ammonia Conversion to Nitrite. Nitrite production data from Figures 6 and 7 were averaged together and replotted as a function of residence time. This presents the first-stage nitrification in a young biomass.

TABLE 3

INFLUENCE OF RESIDENCE TIME ON NITRIFICATION RATE

Residence Time (min)	NO_2-N formed* (mg/ℓ)	NO_3-N formed* (mg/ℓ)	Total Nitrification $NO_2 + NO_3$ (mg/ℓ)	%TKN** Oxidized	Nitrification Rate (mg/hr/g MLVS)***
		UNIT 1			
360	0.0	45.3	45.3	92	1.4
228	0.8	42.6	43.4	87	2.1
139	1.9	32.0	33.5	67	2.7
93	0.8	41.7	42.5	87	5.0
74	0.4	40.7	41.1	85	6.1
		UNIT 2			
228	0.3	44.4	44.7	91	1.65
167	3.0	40.2	43.2	87	2.2
100	0.1	44.1	44.2	89	3.7
78	1.1	39.8	40.9	83	4.5
71	0.4	39.5	39.9	81	4.8

* Each number is the average of five separate runs at the given residence time.

** Unit 1 = 5.5 g MLVS; Unit 2 - 7.1 g MLVS.

*** % Based on average feed of 49.3 mg/ℓ TKN (Table 2).

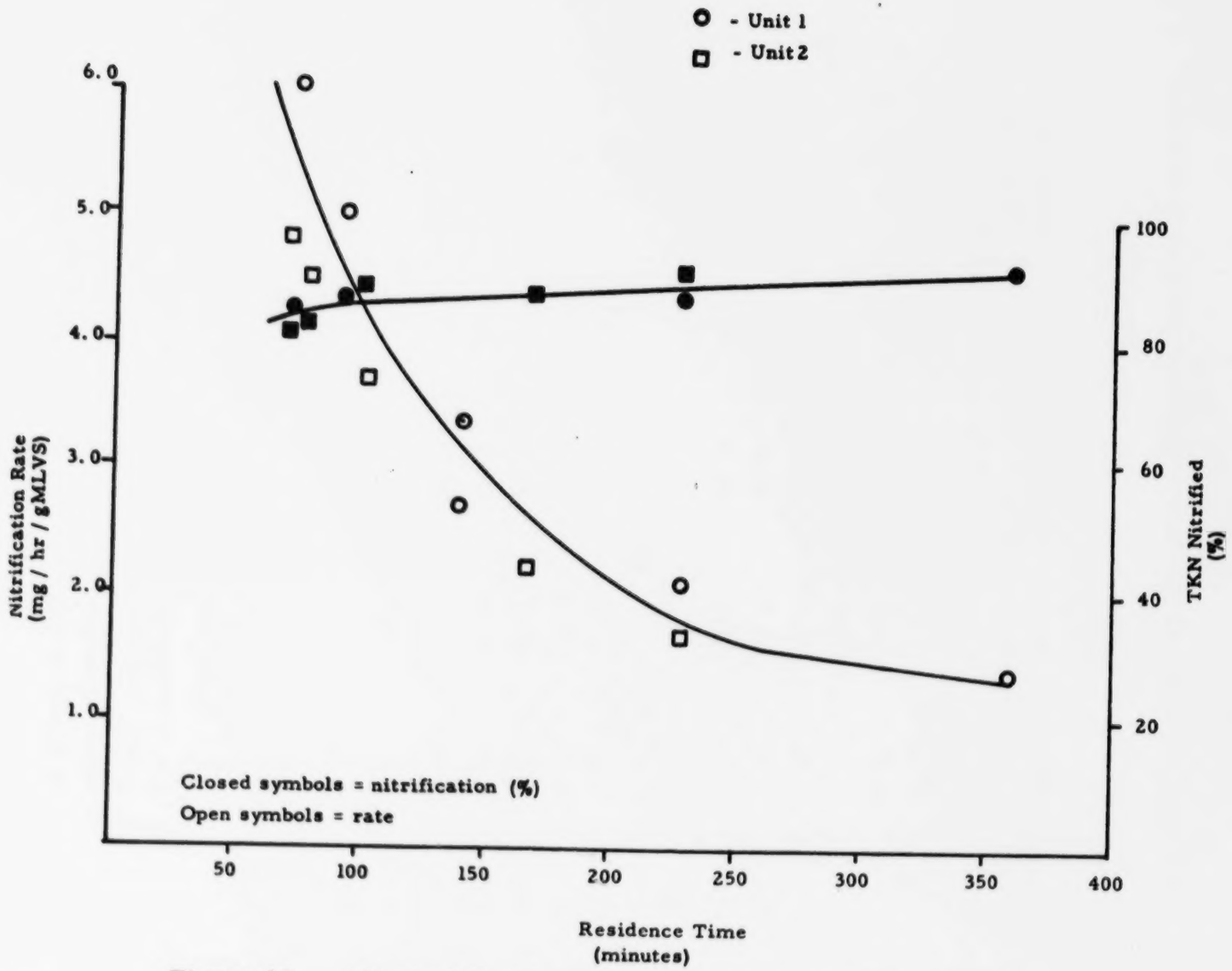


Figure 10. Affect of Residence Time on Rate and Efficiency of Nitrification. Data from Table 3 is plotted to show that at some limiting maximum nitrification rate, the efficiency of the process starts to fall off.

TABLE 4

DRY WEIGHT AND VOLATILE SOLIDS

Sampling Date	Unit 1		Unit 2	
	Total Dry Weight, g	Volatile Material %	Total Dry Weight, g	Volatile Material %
6/13/69	19.6	-	22.3	-
7/2/69	29.5	-	9.7	-
12/18/69	19.4	68.3	22.8	86.5
2/17/70	8.2	74.8	26.7	87.8
Average:	19.2	71.6	20.4	87.2
Average Total Volatile Solids	13.8		17.8	
Average MLVS	5500 mg/l		7100 mg/l	
Average MLVS Combined Units	6300 mg/l			

These beds eventually recover. This effect can also appear if some accident befalls the system such as a complete power failure for prolonged periods. These circumstances are reflected in some of the data of Table 4 but it is presumed (through visual evaluation) that these low levels were transitory and the average solids determinations reflect the true picture. Figures 11, 12, and 13 show the response of the growing microflora during restart as changes in the NH_3-N, TKN, NO_2-N, NO_3-N, and COD levels of the effluent. As noted previously, the fraction of the microflora which oxidizes ammonia to nitrite grows at a faster rate than the fraction which oxidizes nitrite to nitrate. The rate of regrowth of the second group, however, is much more rapid than the two months initially required for their development.

As shown in Figure 11, reduction of ammonia nitrogen in the effluent developed quite rapidly, with maximum conversion occurring after the first day of operation. This immediate reduction in ammonia level is witnessed again as a rapid drop in total Kjeldahl nitrogen (TKN) level. The capacity to oxidize the remaining organic nitrogenous components also measured by the Kjeldahl analysis developed more slowly, as evidenced by the gradual decrease in TKN after the first day of operation.

Nitrite (Figure 12) was the predominant product of ammonia oxidation on the first day, accounting for 75% of influent nitrogen. By the second day of operation, the microflora had oxidized more than half of this nitrite to nitrate. In subsequent continuous operation, nitrate producers presumably form the predominant nitrifying species, judging from the fact that nitrite ultimately appears to be oxidized as rapidly as it is produced.

Recovery of the capacity to remove COD, seen in Figure 13, occurred rapidly until the third day of operation, when effluent quality became stable at a level of 35% of influent COD remaining.

By the third day of operation, the effluent produced by Unit 1 was highly oxidized. Essentially all of the ammonia had been converted to nitrate and nitrite, and COD removal was maximal. In contrast, however, the bed microflora at this point consisted of a relatively thin slime film covering the packing rings. Development of a typically dense, luxuriant bed growth required an additional 7 to 10 days of operation. It is apparent then that the initial sparse flora which develops is capable of a high rate of metabolism utilizing the majority of available nutrients. The capacity of the bed to oxidize less available or more resistant compounds develops more slowly. Maximum oxidation of organic nitrogen required 6 to 8 days of operation, as did maximum levels of conversion of nitrite to nitrate.

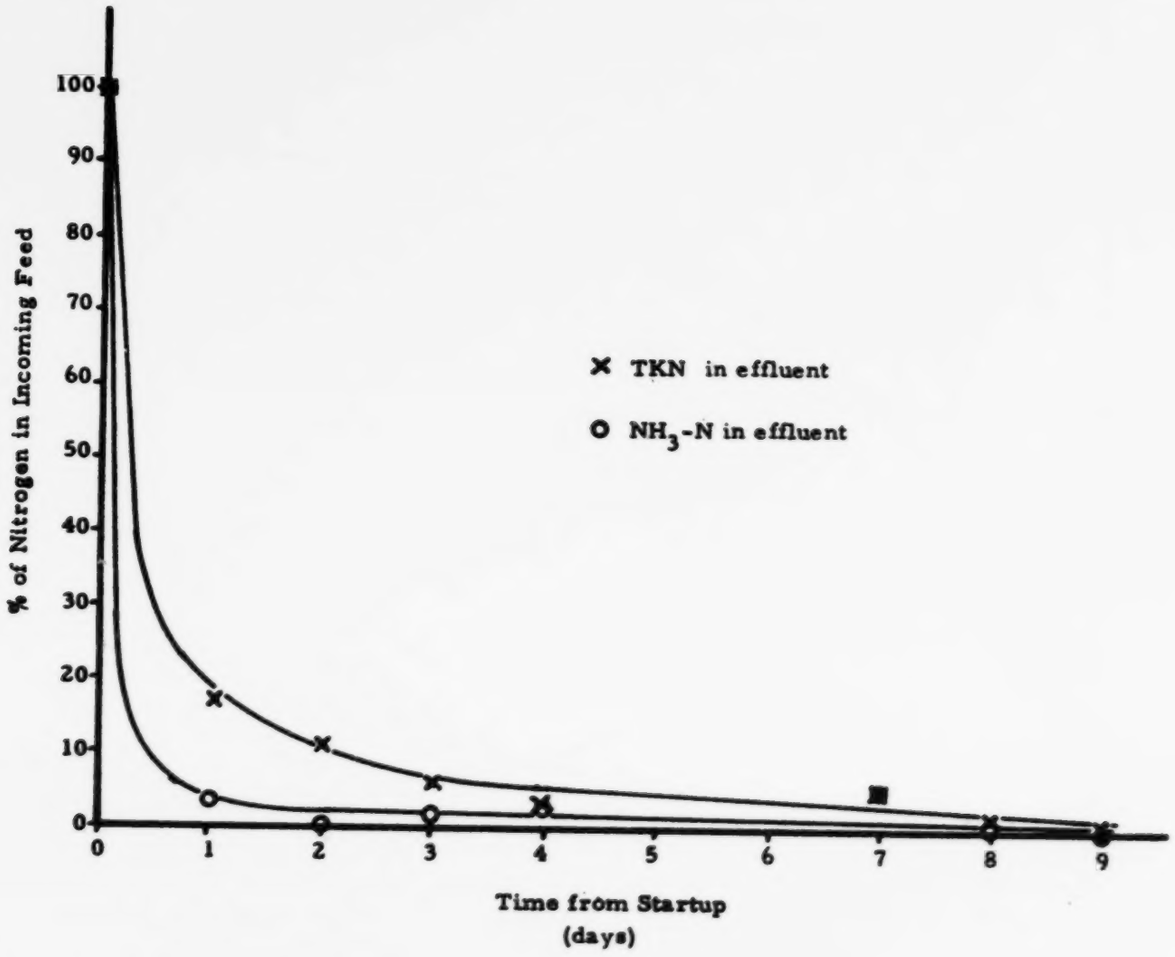


Figure 11. Development of Ammonia Oxidizing Ability in a Freshly Assembled Unit - Run 7869. Sewage influent 15 ml/min (mean residence time = 167 min); 29°C. Unit 1 had been disassembled and most of the biomass rinsed off of the walls and rings prior to the start of this run.

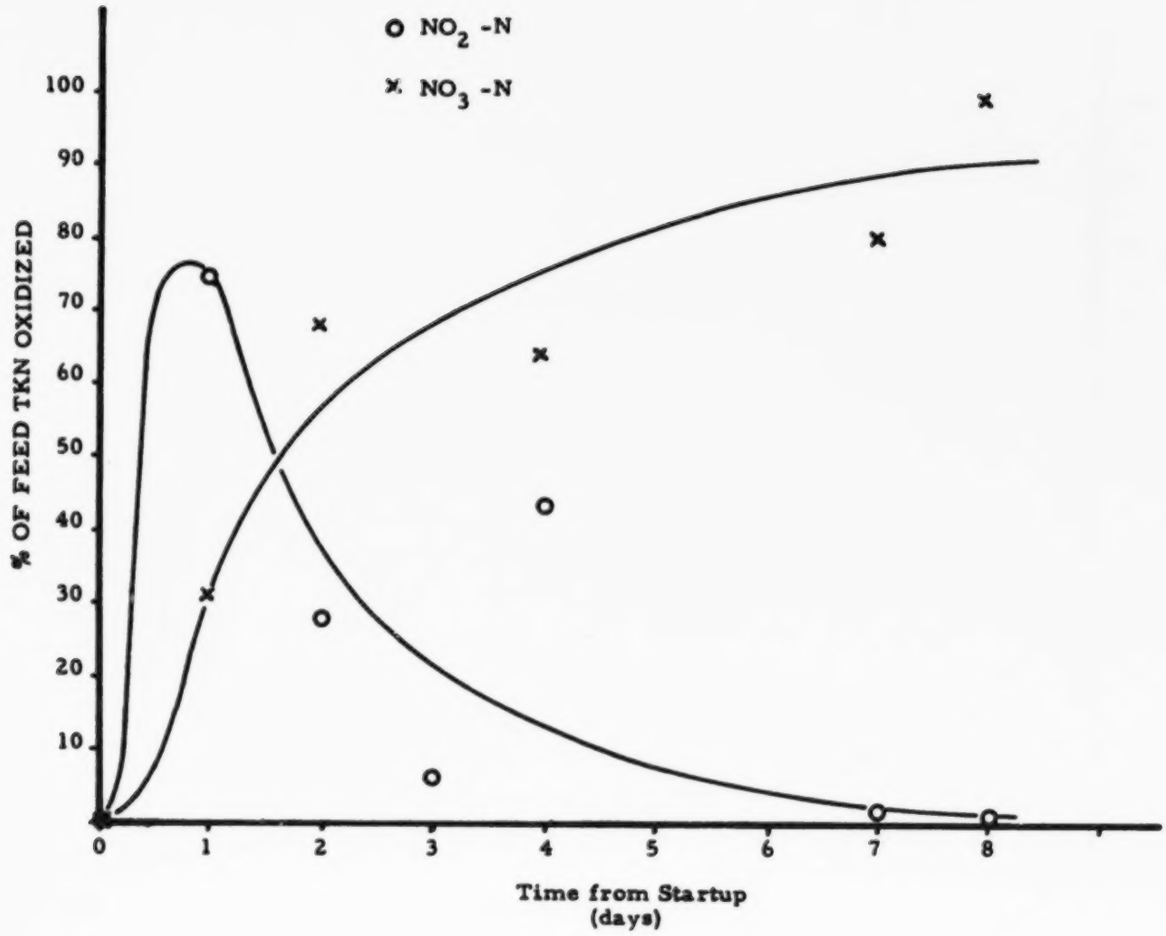


Figure 12. Development of Nitrifying Ability in a Freshly Assembled Unit - Run 7869. Influent 15 ml/min (mean residence time = 167 min); 29°C. Unit 1 had been disassembled and most of the biomass rinsed off of the walls and rings prior to the start of this run.

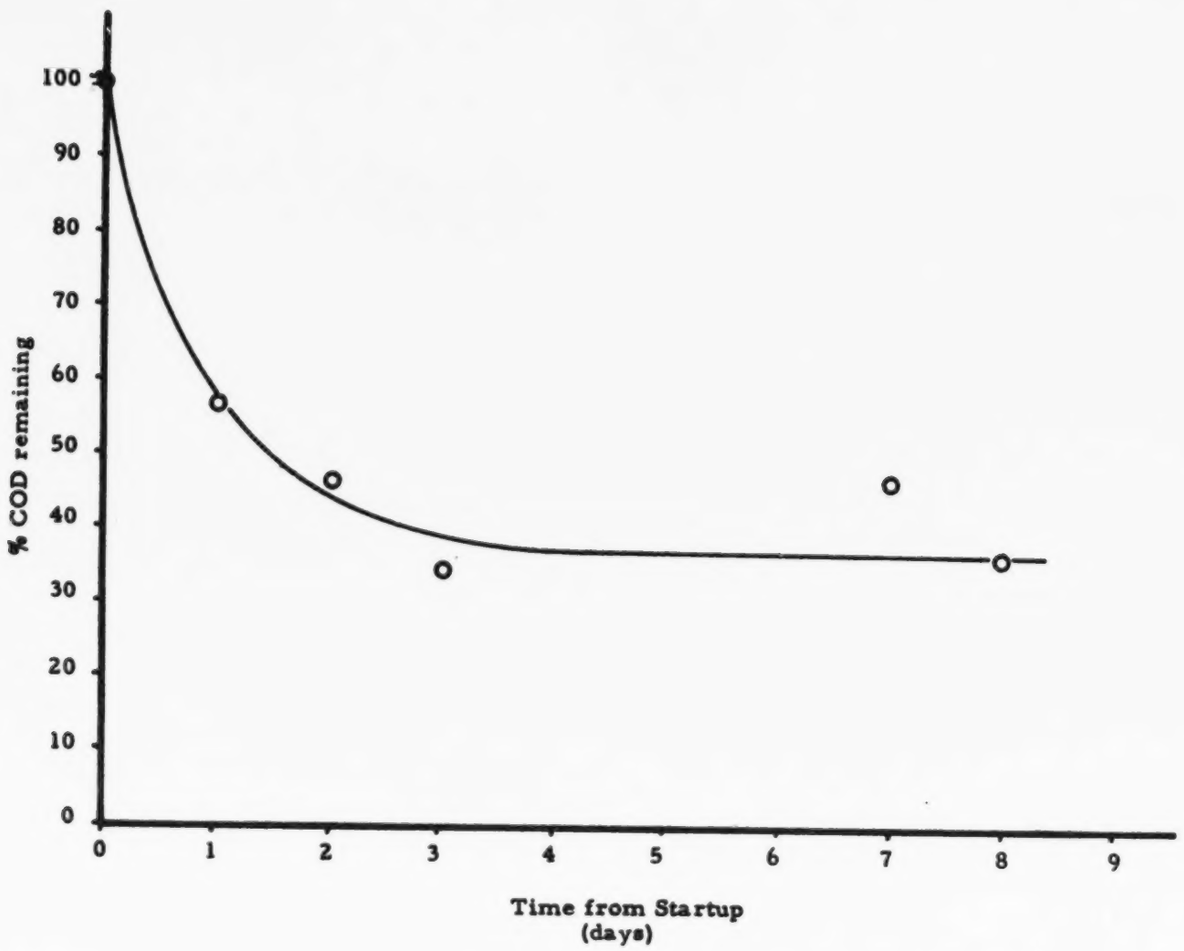


Figure 13. Development of COD Oxidizing Ability in a Freshly Assembled Treatment Unit - Run 7869. Influent 15 ml/min (residence time = 167 min); 29°C. Unit 1 had been disassembled and most of the biomass rinsed off of the walls and rings prior to the start of this run.

VI. DENITRIFICATION

BATCH PROCESSING OF NITRIFIED EFFLUENTS

With the conditions that control nitrification in the Activated Bed units defined, there was now available a source of highly oxidized sewage effluent for the denitrification experiments. The intent of this first phase of the denitrification studies was to determine if the stored food reserves of the microflora were indeed capable of supporting the reduction of the nitrates. To avoid the complications introduced by dilution and overflow in a continuously flowing system, the initial studies were carried out on a batch basis.

The two units were aerobically stabilized at a residence time (70 min) that provided a completely nitrified effluent using Azusa primary effluent as feed. Temperature was carefully controlled at 29.0°C and COD, NH_4-N, NO_2-N, and NO_3-N were analyzed daily for a period of 14 days to insure that an equilibrium situation between feed and effluent had been established and a stable biomass was present.

The denitrification experiments were carried out by turning off the influent feed pumps and cutting off the air supply. The recirculating liquor was then monitored versus time to determine the fate of the various nitrogen compounds. Opportunity was also afforded, in these experiments, to follow O_2 utilization and release of amino acids and proteins into the medium. These latter results will be discussed in subsequent sections.

Figure 14 presents one of the early experiments in this series. The purpose was to determine if all of the nitrate contained in the circulating liquor could be biologically converted. In a matter of less than 24 hrs there was no detectable nitrate left in the system.

Figures 15 and 16 are representative of experiments intended to follow changes in the nitrogen compounds of interest. Only traces of NO_2^- were present and therefore these are not shown in the figures. An interesting observation was the steady rise in ammonia nitrogen. This indicates utilization of the amino acid pools of the microflora as anaerobiosis progresses and does not originate from the NO_3-N in the recirculating liquor. This low level of ammonia was also observed in the continuous experiments that will be discussed later.

The denitrification rates for the batch experiments at 29°C ranged from 0.39 - 0.89 mg/hr/g MLVS with a combined average rate for the two units of 0.58 mg/hr/g MLVS (Table 5). Low temperature data will be discussed in the following section.

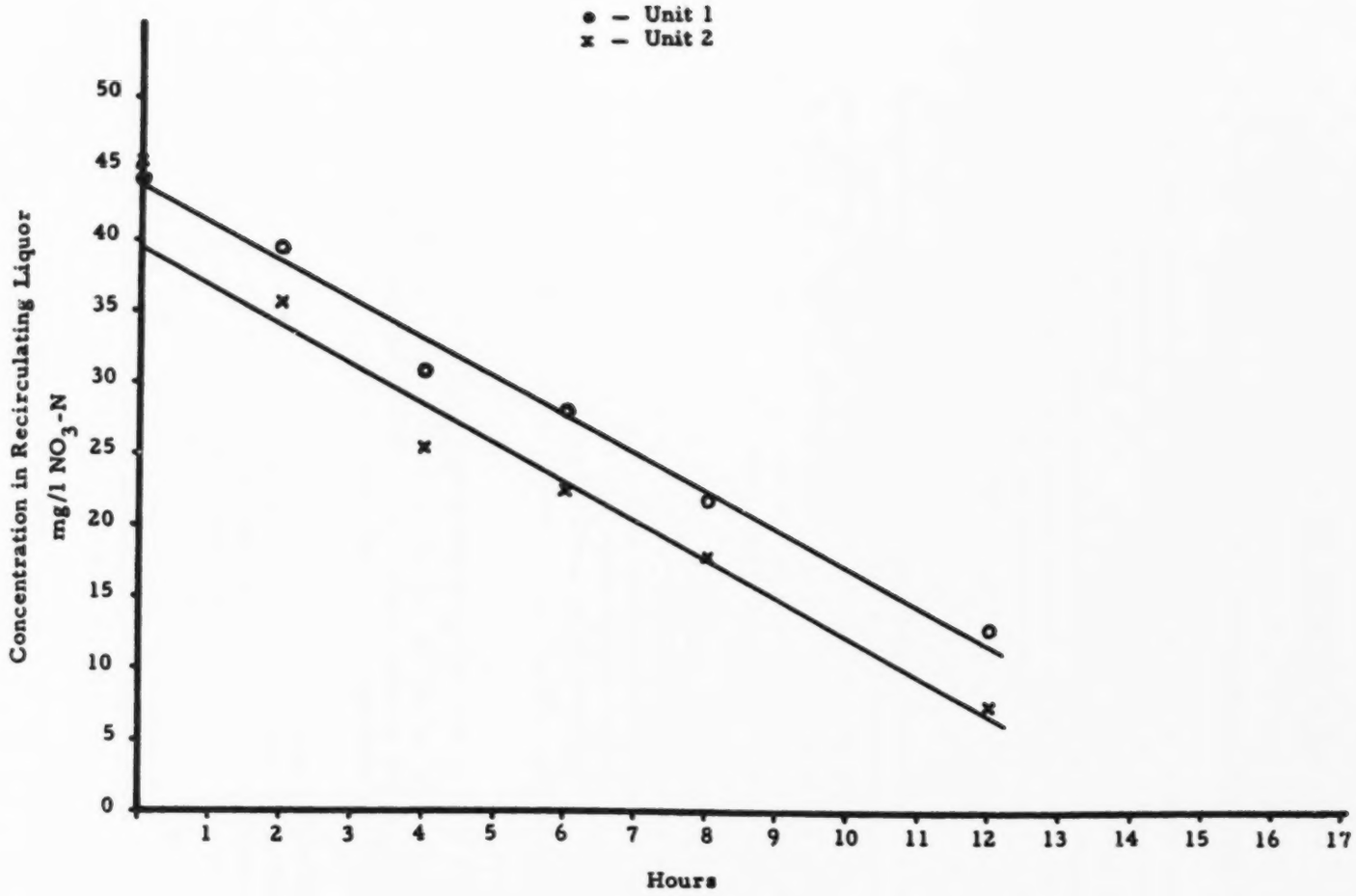


Figure 14. Ability of Activated Bed unit to Denitrify as a Batch Process - 29°C. Run 10969. Air supply and feed to actively nitrifying units were both turned off at time = 0. The decrease in NO_3^- in the recirculating liquor was then followed. Denitrification rate, R = 0.49 mg/hr / g MLVS.

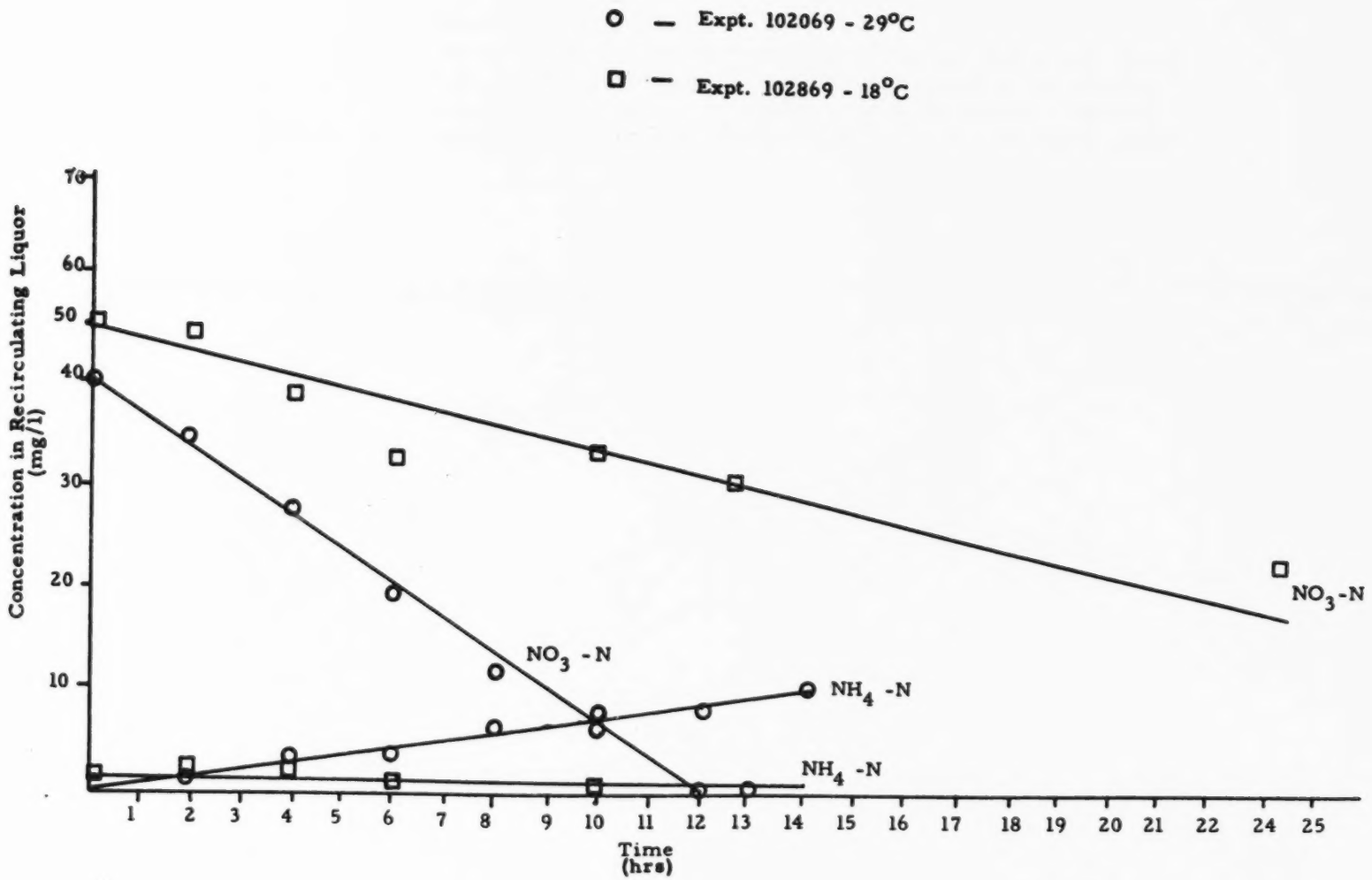


Figure 15. Principal Nitrogen Species Present During Batch Denitrification - Effect of Temperature on Rates - Unit 1. Air supply and sewage feed to actively nitrifying units turned off at t = 0. Units acclimated to temperature one week prior to start of run.

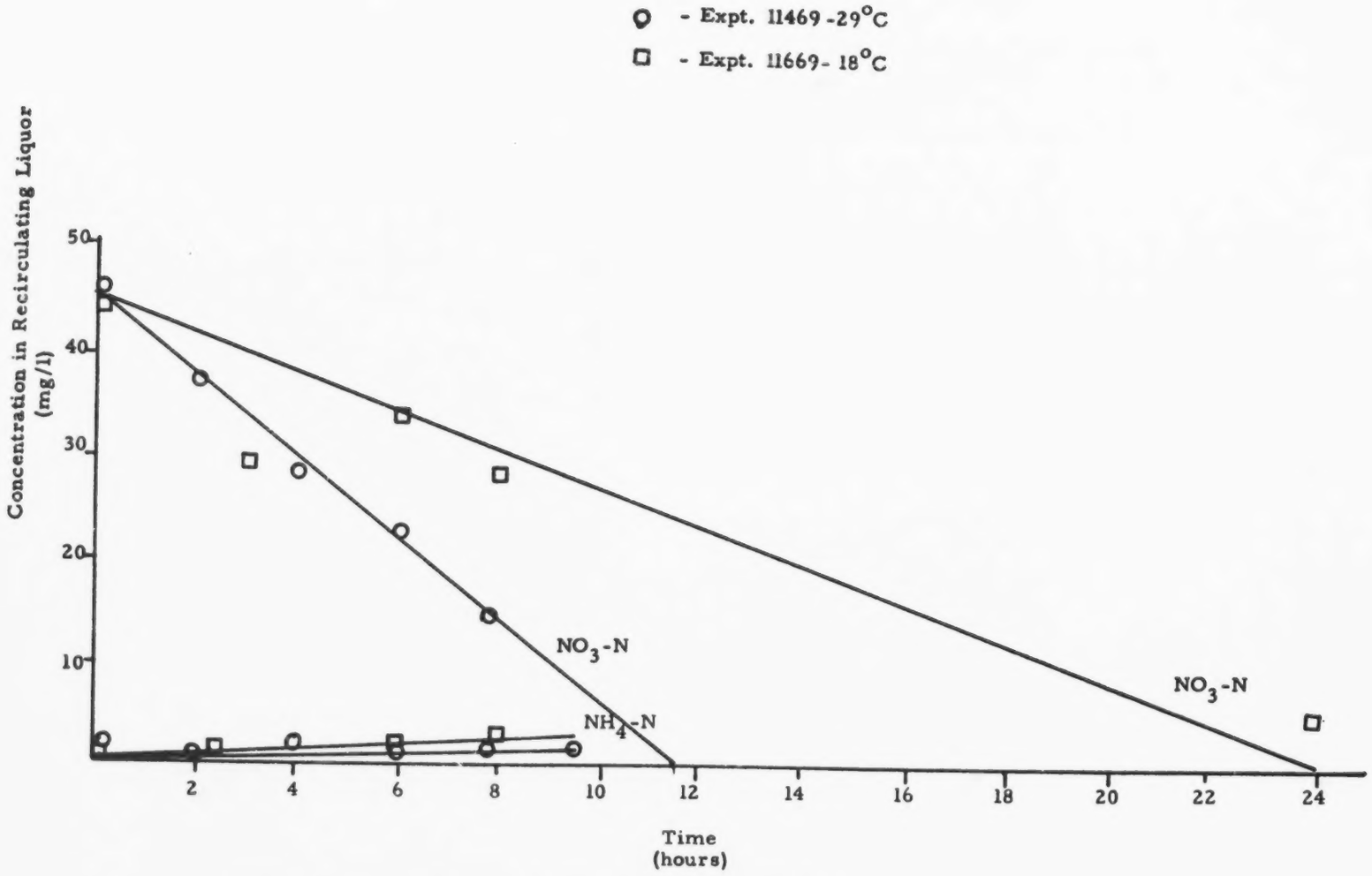


Figure 16. Principal Nitrogen Species Present During Batch Denitrification - Effect of Temperature on Rates - Unit 2. Air supply and feed to actively nitrifying units turned off at t = 0. Units acclimated to temperature one week prior to start of run.

TABLE 5

SUMMARY OF BATCH DENITRIFICATION RATES
(29°C)

Experiment	NO_3-N Start (mg/ℓ)	NO_3-N End (mg/ℓ)	Time Span (hrs)	NO_3-N Removed (mg/hr)	Removal Rate (mg/hr / g MLVS)*
			UNIT 1		
10969	44.0	17.0	10.0	2.7	0.49
102069	40.8	6.4	10.0	3.4	0.62
102369	39.2	24.0	6.0	2.5	0.45
11469	48.8	26.4	7.8	3.4	0.62
			UNIT 2		
10969	40.0	28.0	10.0	2.8	0.39
102069	40.4	15.2	4.0	6.3	0.89
102369	38.0	11.2	6.0	4.4	0.62
11469	46.4	14.4	7.8	4.1	0.58

Average Rate (both Units) = 0.58 mg/hr/g MLVS

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* MLVS - used for calculations were the average values from Table 4.
Unit 1 = 5.5 mg/ℓ ; Unit 2 - 7.1 mg/ℓ.

Temperature Effects on Denitrification Rate

A group of batch experiments were initiated to obtain some preliminary information on the effects of temperature on denitrification rate. This series was preceded by an acclimation of the microflora for a period of one week at 18°C under aerobic conditions. Residence time was adjusted to 83 min for the aerobic phase preceding the batch denitrification run, to assure a well nitrified recirculating liquor.

Figures 15 and 16 are typical of these runs. It is quite apparent that the rates are much slower than at 29°C. The 18°C runs are presented in Table 6. The range for these runs is 0.21 - 0.34 with an average rate for the combined units of 0.26 mg/hr/g MLVS. These values will be further refined and more fully discussed in the section dealing with continuous operation.

CONTINUOUS FLOW-THROUGH AND DENITRIFICATION

The work to this point demonstrates that mature activated bed units are capable of complete denitrification of secondary effluent without the addition of external energy sources. Up until now only semi-batch studies have been reported and it is necessary to demonstrate denitrification during continuous operation. Information reported in this set of experiments will then be used to predict overall denitrification when the system was cycled in a coupled mode. To enable the prediction of the equilibrium nitrate level, and to determine an optimum time for recycling, a mathematical model of our system's performance was developed and its predictions tested by operating the units under various test conditions.

The Nitrification-Denitrification Model - A Mathematical Interpretation

A complete derivation of a model describing nitrification-denitrification in which all parameters are considered to vary with time is not only extremely difficult, but unnecessary at this point. An unnecessarily complicated model also obviates easy comparison of actual experimental data with the data predicted from the model.

In order to construct a model that would be useful in guiding the experimental program, several simplifying assumptions were made. The derivation assumes: (1) that the nitrogen composition of the primary effluent feed to the Activated Bed units is constant and at 40-50 mg/ℓ ammonia nitrogen (NH_3 -N), negligible nitrate nitrogen (NO_3 -N), and negligible nitrite nitrogen (NO_2 -N), and (2) that under anaerobic conditions a given biomass in one unit denitrifies at a constant rate, as ascertained in the batch denitrification studies. The final refined form of the model takes into account all predominant inorganic species containing nitrogen. The following symbols are used in the derivation and model:

TABLE 6

SUMMARY OF BATCH DENITRIFICATION RATES

($18^{o}C$)

Experiment	NO_3-N Start (mg/ℓ	NO_3-N End (mg/ℓ)	Time Span (hrs)	NO_3-N Removed (mg/hr)	Removal Rate (mg/hr / g MLVS)*
			UNIT 1		
102869	46.4	30.4	12.7	1.26	0.23
11669	44.0	16.4	24.0	1.15	0.21
111769	42.0	31.0	7.5	1.47	0.27
			UNIT 2		
102869	40.4	18.0	12.7	1.76	0.25
11669	45.6	4.8	24.0	1.70	0.24
111769	44.0	3.0	17.0	2.40	0.34

Average Rate (both units) = 0.26 mg/hr / g MLVS

* MLVS - used for calculation were the average values from Table 4. Unit 1 - 5.5 mg/ℓ ; Unit 2 - 7.1 mg/ℓ.

$Y(T, t)$	=	total inorganic nitrogen concentration (mg/ml) (i. e., NO_3-N, NO_2-N, NH_3-N) in the anaerobic effluent as a function of time and temperature
R	=	constant rate at which biomass removes inorganic nitrogen (mg/min)
$R(T)$	=	$R_{NO_3}(T) - R_{NH_3}(T)$ = total rate of disappearance of inorganic nitrogen as function of temperature (mg/min)
N_f	=	total inorganic nitrogen in aerobic feed (Azusa primary effluent)
t	=	time (minutes)
T	=	temperature (^oC)
N^t	=	total inorganic nitrogen concentration in anaerobic unit at switching time, t
Q	=	anaerobic system volume (ml)
v	=	aerobic system volume (ml)
F	=	feed flow of nitrified liquor to an anaerobic system (ml/min)
f	=	feed flow of sewage to aerobic system (ml/min)
$c(t)$	=	total inorganic nitrogen concentration in aerobic system as a function of time

Also let $F/Q = a$, $f/v = b$.

From batch run denitrification results, as illustrated in Figures 14, 15, and 16, rates of nitrate removal and ammonia buildup at 18°C and 29°C were measured. Changes in nitrite nitrogen were found to be insignificant. For the Activated Bed system between 18°C and 29°C, experimental rates were:

$$R_{NO_3}(T) = -0.40 + 0.035\ T \text{ (mg } NO_3\text{-N/min)}$$

$$R_{NG_3}(T) = -0.062 + 0.0073\ T \text{ (mg } NH_3\text{-N/min)}$$

$$R(T) = R_{NO_3}(T) - R_{NH_3}(T) = -0.34 + 0.028\ T \text{ (mg N/min)}$$

describes the temperature dependence of the rate of disappearance of all inorganic nitrogen during anaerobiosis.

Appendix II gives a complete mathematical derivation of the model. The resulting solutions are:

$$Y(t, T) = N_f + \left(\frac{-0.34 - 0.028\,T}{F}\right)\left(1 - e^{-a(t-t_1)}\right) + \frac{a\,(\Delta N^{t_1})}{2-b}\left[e^{-b(t-t_1)} - e^{-a(t-t_1)}\right] \qquad (1)$$

and

$$c(t) = (\Delta N^{t_1})\; e^{-b(t-t_1)} + N_f \qquad (2)$$

The rate of nitrification in the aerobic Activated Bed units was assumed to be very rapid and thus unimportant when compared to nitrogen changes caused by simple dilution affects.

A computer program for solution of this equation was written and is stored in Aerojet-General's computer Engineering Terminal System, to facilitate rapid data analysis.

A closer look at the behavior of these solutions yields several interesting features. If, for example, at t_o Unit 2 is switched to an anaerobic mode, and the system is left in this mode (i.e., no cycling), the nitrate concentration in Unit 2 will theoretically stabilize at:

$$\lim_{t \to \infty} y(t) = \lim_{t \to \infty} \frac{N_f F - R}{F}\left(1 - e^{-(F/Q)t}\right) + N_f\, e^{-(F/Q)t}$$

from equation (1)

$$\lim_{t \to \infty} y(t) = N_f - \frac{R}{F} \qquad (3)$$

In fact, the stabilized level is the same for an anaerobic unit if the cycling is stopped at some point, the unit remains anaerobic, and nitrified feed continues at a steady rate. This assumes that the microflora does not become energy deficient. Thus, if the experimental nitrate concentration in the anaerobic unit is observed to be changing with time at large t (i.e., t ∞, approx.) and N_f and F are held fixed, then R must be changing. Since, in particular, equation (1) for y(t) is monotonically decreasing, any increase in nitrate concentration at large times can only be attributed to a decrease in R, the denitrifying rate of the biomass. Thus,

$$\lim_{t \to \infty} y(t) = N_f - \frac{R}{F}$$

may be a useful tool in evaluating when and how the denitrifying ability of the biomass fails with extended anaerobiosis. The discussions of the experimental results will demonstrate the usefulness of the model in understanding the operation of the units.

Coupled Operation and Control of Denitrification

The feed rate to the denitrification unit could now be calculated to give any desired denitrification efficiency. Using the equation derived from the model:

$$F = \frac{R}{(N_f - Y_e)} \qquad (4)$$

(where Y_e is the desired equilibrium level in the anaerobic effluent).

Figures 17 and 18 show the results from the experiments in which the above equation was used to set the flow rates for complete denitrification. In these runs Activated Bed Unit 2 was in the anaerobic mode and was receiving a highly nitrified feed from Activated Bed Unit 1.

The graphs show that the nitrified recirculating liquor contained in the anaerobic Activated Bed units at time zero is simultaneously denitrified and diluted. Nitrates in the liquor continue to decrease until the equilibrium point between feed rate and denitrification rate is reached. The apparent jump in NO_3 at the end of the experiment in Figure 17 is not yet understood but it is indicative of a change in denitrification rate late in the experiment.

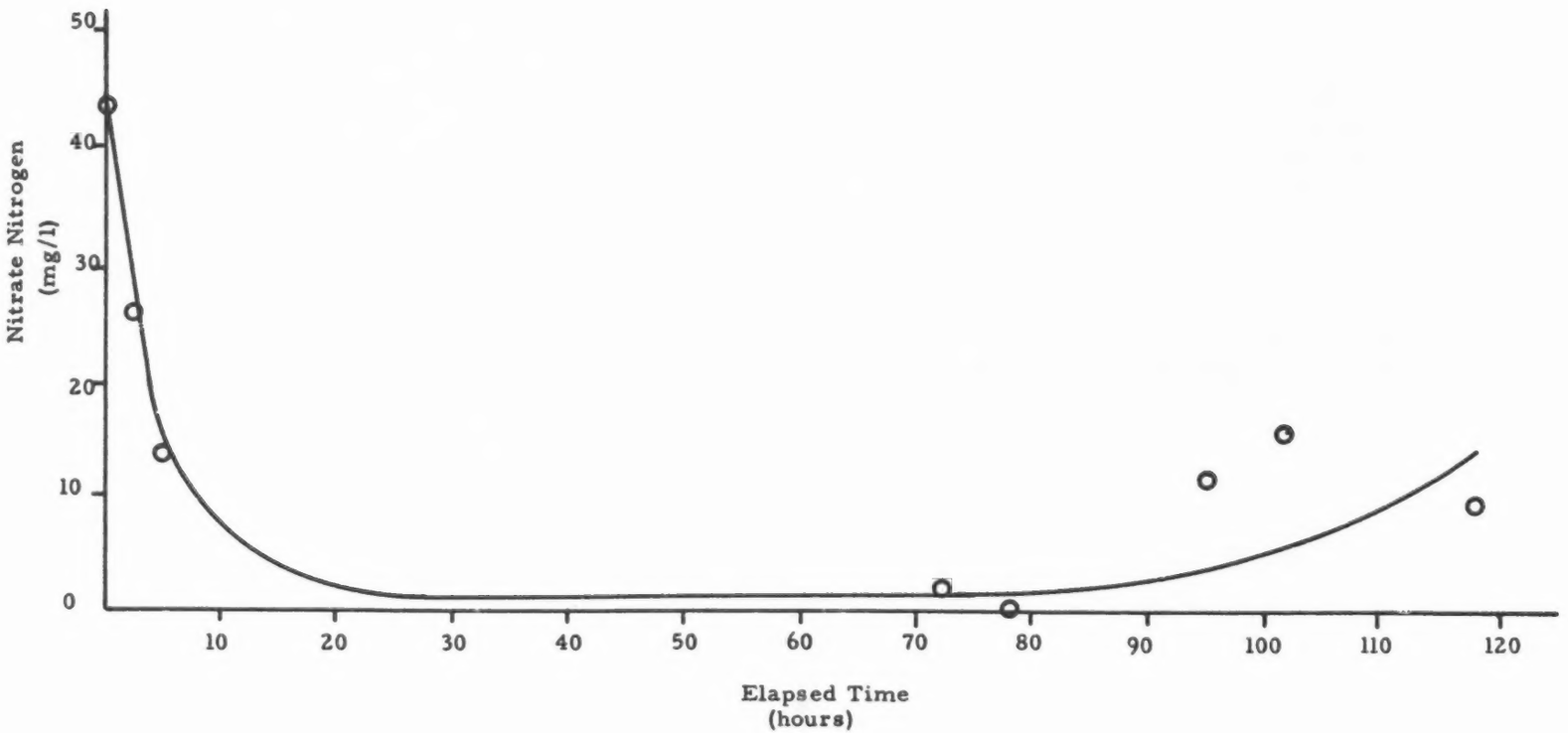


Figure 17. Decrease in NO_3-N in Effluent as a Function of Simultaneous Dilution and Denitrification - Unit 2, Run 111769, Phase c, 18°C. Air supply and feed to the actively nitrifying unit was turned off and the oxidized effluent from Unit 1 was metered in at 2.5 ml/min (residence time - 1000 min).

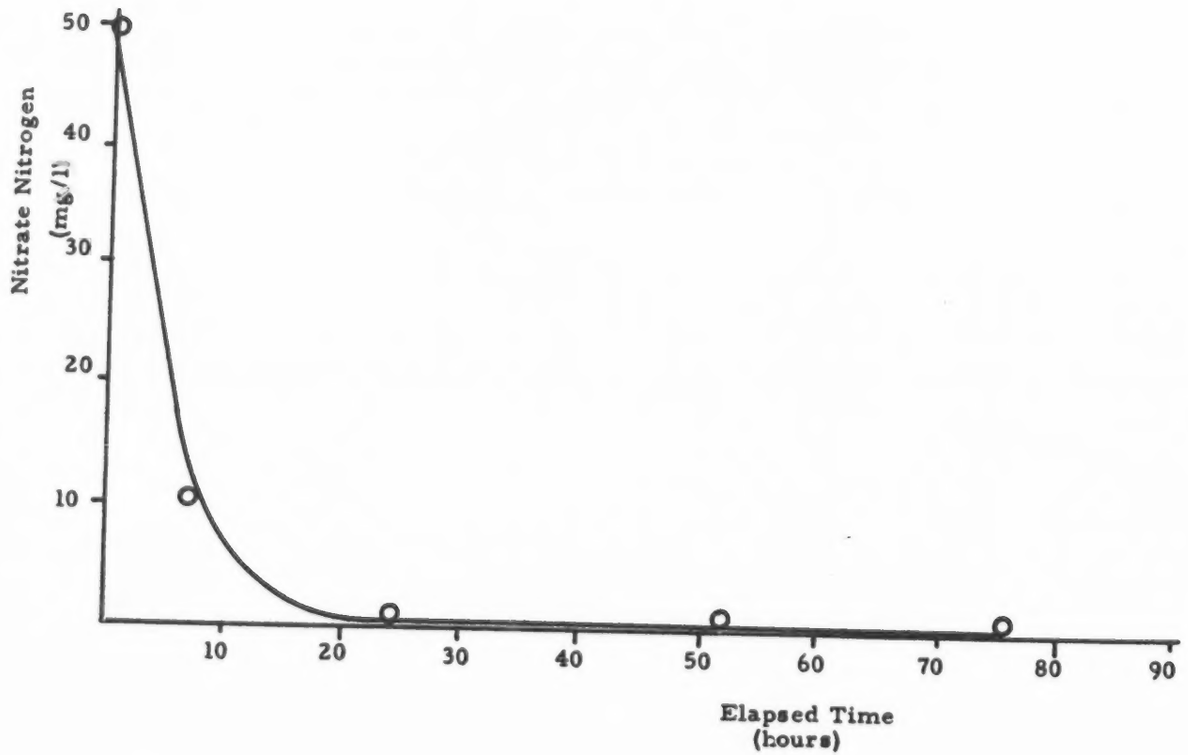


Figure 18. Decrease in NO_3-N in Effluent as a Function of Simultaneous Dilution and Denitrification - Unit 2, Run 1570, Phase d, 29°C. Air supply and feed to the actively nitrifying unit was turned off and the oxidized effluent from Unit 1 was metered in at 2.5 ml/min (residence time - 1000 min).

VII. CYCLED OPERATION OF THE DENITRIFICATION SYSTEM

The system model proved to be extremely useful in understanding the inorganic nitrogen levels in the Activated Bed units during coupled aerobic-anaerobic cycling, which as a major objective of this study. The model was shown to successfully predict nitrogen levels in both units during coupled operation. It was useful in establishing physical parameters of the system when a particular output of nitrogen was desired in the anaerobic effluent. Moreover, the model pointed out physiological features of the microflora, such as denitrification rate changes during an extended anaerobic phase, and denitrification rate dependence on temperature in cycled operation.

A series of cycling experiments were carried out to determine the effects of alternating the microflora between the aerobic and anaerobic modes. The units were kept at constant temperature and were fed continuously throughout the experiments. A schematic description of this mode of operation is provided in Figure 19. The assumptions used in deriving the model were checked by calculating a predicted result for each of the sets of experiments.

The first series of experiments was initiated by cutting off air and feed flows to Activated Bed Unit 2 and letting it denitrify as in the batch experiments. This was used to establish the rate of denitrification - R. Once this rate was determined the oxidized effluent from Unit 1 was fed into the anaerobic unit at a rate derived from the model.

The initial aerobic-anaerobic cycling experiments were carried out at 18°C. The reason for this was that the culture was well acclimated to this low temperature having just been used in the 18°C batch studies. At the time the air was turned off in Unit 2 at the start of Run 111769, the recirculating liquor contained 44 mg/ℓ of NO_3-N. This decreased to 2.4 mg/ℓ in 17 hrs at which point the feed pump supplying the nitrified effluent was restarted. All of this was done to avoid the complication of dilution in determining denitrification rate if the feed pump had not been turned off at the start. As experience was gained in using the model, this maneuver was no longer necessary. Denitrification rates could be determined at any point in time.

In each experiment the occurrence of a switch in modes (i.e., aerobic to anaerobic) was called a phase switch. Each phase was then timed from the point of switching to enable readily monitoring the number of consecutive hours a unit had been anaerobic.

Returning now to Run 111769, the denitrification rate was calculated as 0.340 mg/hr / g MLVS (Table 6; also Table 7, Phase a). The feed pump at 17 hours was adjusted to a flow rate of 2.5 ml/min which gives a 1000 minute residence time in the Activated Bed units. On a continuous feed basis, the rate of denitrification should stabilize at some constant level of nitrogen removal. This point was established

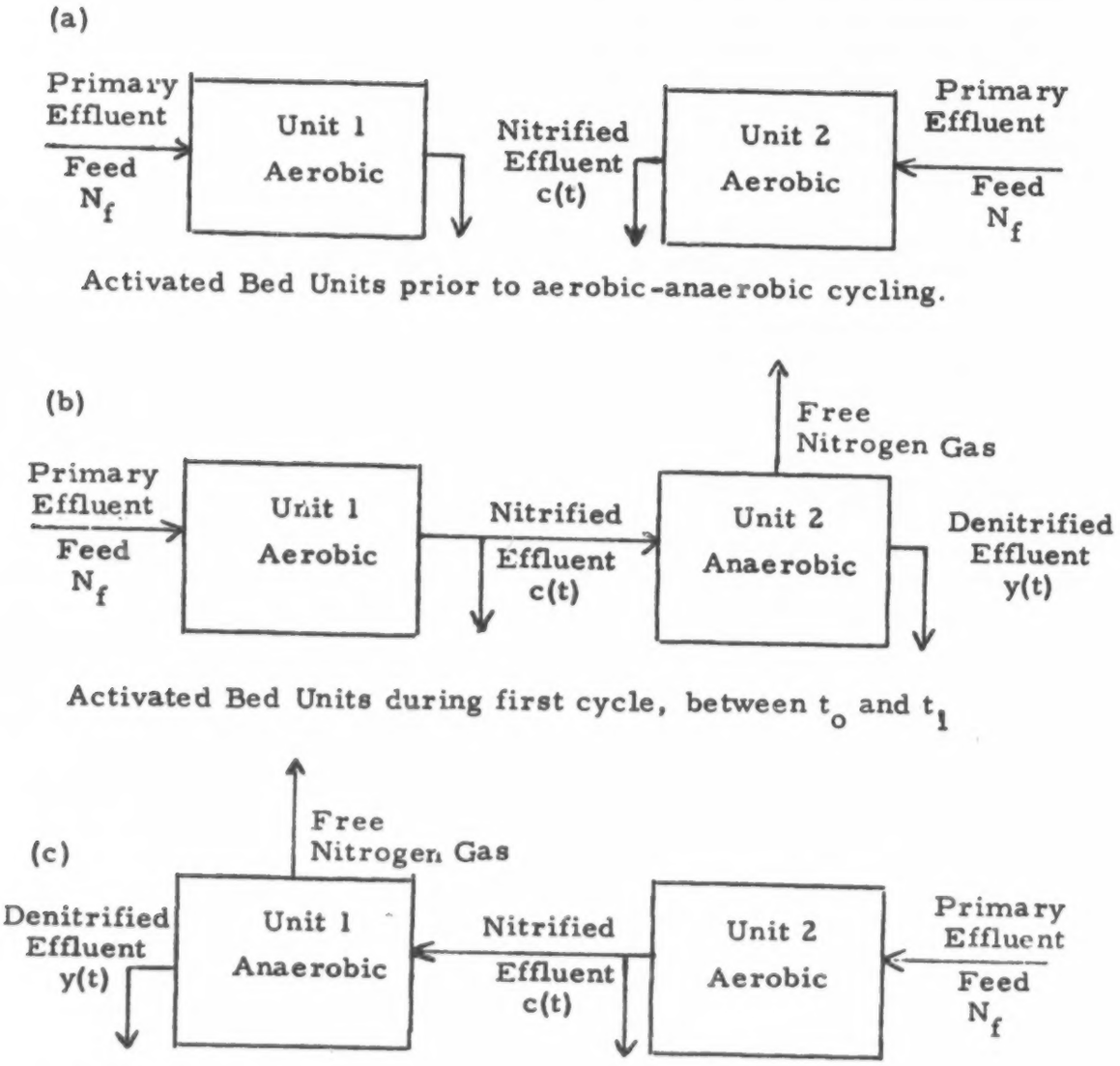


Figure 19. Schematic Illustrating Coupled Operation of the Nitrification-Denitrification System

TABLE 7

CONTINUOUS RUN DENITRIFICATION RATES AT 18°C

Run No.	Phase*	Elapsed Hours from Phase Switch	R = Rate (mg/hr / MLVS) Unit 1	Unit 2
111769	a	22.7		.350
		24.5		.392
		31.0		.338
		40.7		.280
	b	13.2	.291	
		24.2	.282	
		28.7	.282	
		32.7	.218	
		47.2	.273	
	c	3.0		.651
		5.5		.742
		72.0		.323
		78.0		.338
		95.5		.252
		101.5		.216
		117.5		.270
121569	a	25.5		.310
		31.5		.334
		50.0		.350

* Occurrence of a switch in modes (i.e., aerobic to anaerobic) is called a phase switch. Each phase was then timed from the point of switching.

at approximately 87% of the incoming nitrogen being removed resulting in a denitrification rate of 0.28 mg/hr / g MLVS (Table 7, Phase a). The sequence of events is illustrated in Figure 20.

The actual data as shown in Figure 20 obtained in Experiment 111769 is plotted along with the values predicted by the mathematical model. It is observed that, except for one point (a), which will be discussed later, there is excellent agreement between the two curves.

At 40.7 hrs, Unit 2 was returned to the aerobic mode and its recovery compared to that predicted by the model. The return to full nitrifying capacity is rapid, but does not precisely fit the predicted curve. Reference will be made again to this fact when we discuss Unit 1.

Also at the 40.7 hr mark Unit 1 was switched from the aerobic to the anaerobic mode (Figure 21). In this case the batch mode was continued only long enough to permit calculating the initial denitrification rate of 0.27 mg/hr / g MLVS (Table 6). At 49 hrs, nitrified effluent was fed in at 2.5 ml/min (1000 min residence time). In this mode (Table 7, Phase b), the equilibrium rate was 0.273 mg/hr / g MLSS. Although the rates for the two units are very similar the actual % nitrate removals differ (87%, Unit 2; 51%, Unit 1) as a consequence of the lower MLVS in Unit 1.

As with Unit 2, except for an anomaly at (b), the experimental results behaved as predicted by the model. Both anomalies (a) and (b) in Figures 20 and 21 can be explained on the basis of the mechanics at the moment of switching from batch to continuous anaerobic operation. In Figure 20, the momentary increase in nitrate nitrogen at (a) corresponds exactly to an opposing decrease in the level of ammonia nitrogen, which drops from 8.7 mg/ℓ at 20 hrs to 1.0 mg/ℓ at 22.7 hrs (Table 8), then rises again to 8.5 mg/ℓ two hrs later. It is probable that the increase in nitrate might have been due to a rise in the dissolved oxygen level of the liquor in the anaerobic unit due to air trapped in the hoses from the pumps, and resulting in either oxidation of ammonia to nitrate or inhibition of denitrification.

At 49 hrs, the feed pump to Unit 1 (Figure 21) was similarly restarted to continuous anaerobic operation. The anomaly at (b) is probably due to similar circumstances. Whatever the real reason for the jump in nitrate, it is apparent that a physiological disturbance occurred. These experiments suggest that even though a low level of incoming dissolved oxygen does not disturb the system - the recirculating oxygen quickly dropping to below 0.5 mg/ℓ, a constant level of residual oxygen may have a detrimental effect.

In figure 20 starting at 41 hrs and in Figure 21 starting at 90 hrs, the behavior of anaerobic units when returned to aerobiosis and a wastewater feed is plotted. Once the air supply is turned on there is an almost immediate recovery in nitrifying ability. As the primary

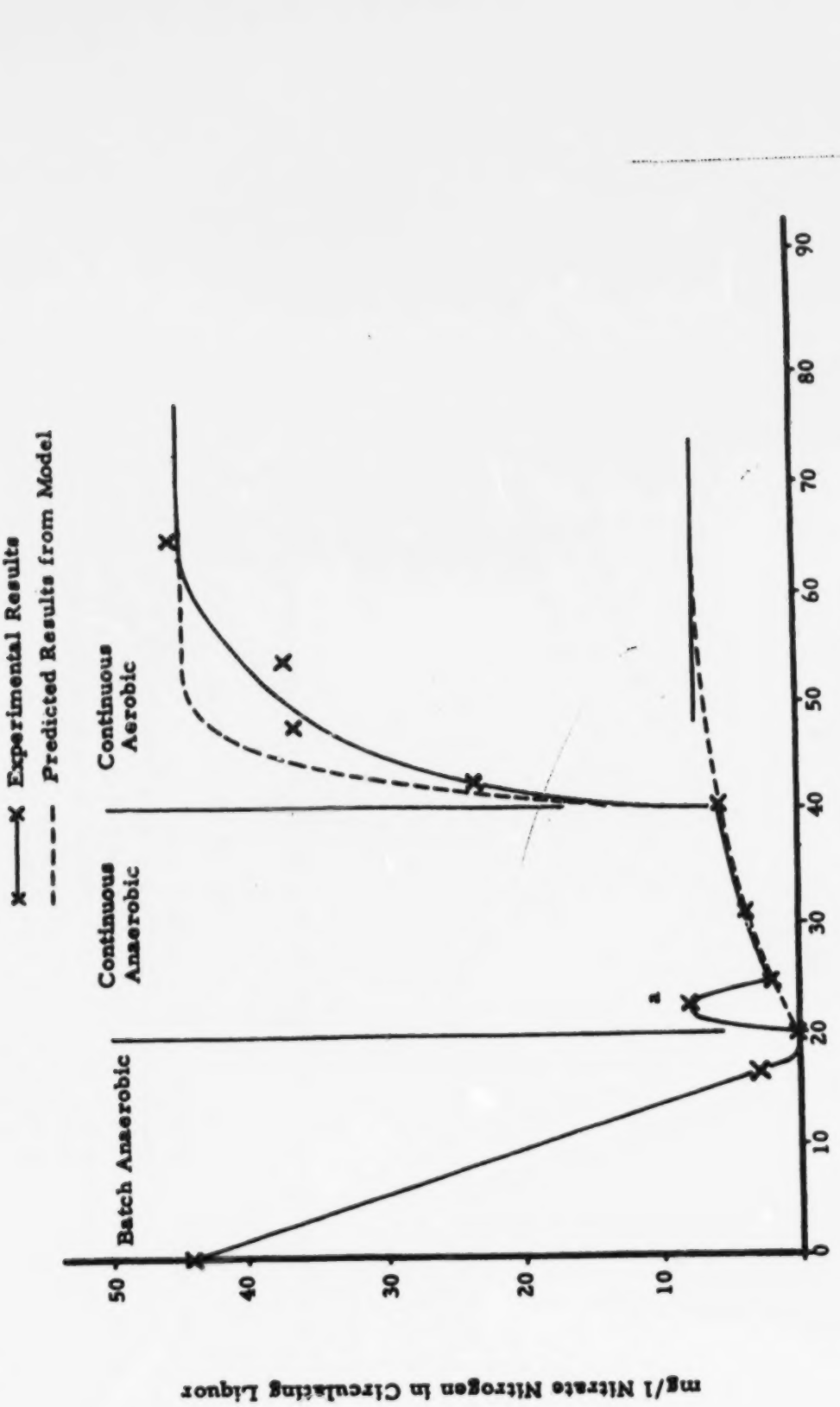


Figure 20. Aerobic-Anaerobic Cycling; A Comparison of Experimental and Predicted Results. Unit 2, Run 111769; 18°C. Batch anaerobic run to obtain an initial denitrification rate for use in the model and to eliminate NO_3 in the recirculating liquor preceded continuous operation. Continuous feed flow set at 2.5 ml/min (residence time = 1000 min).

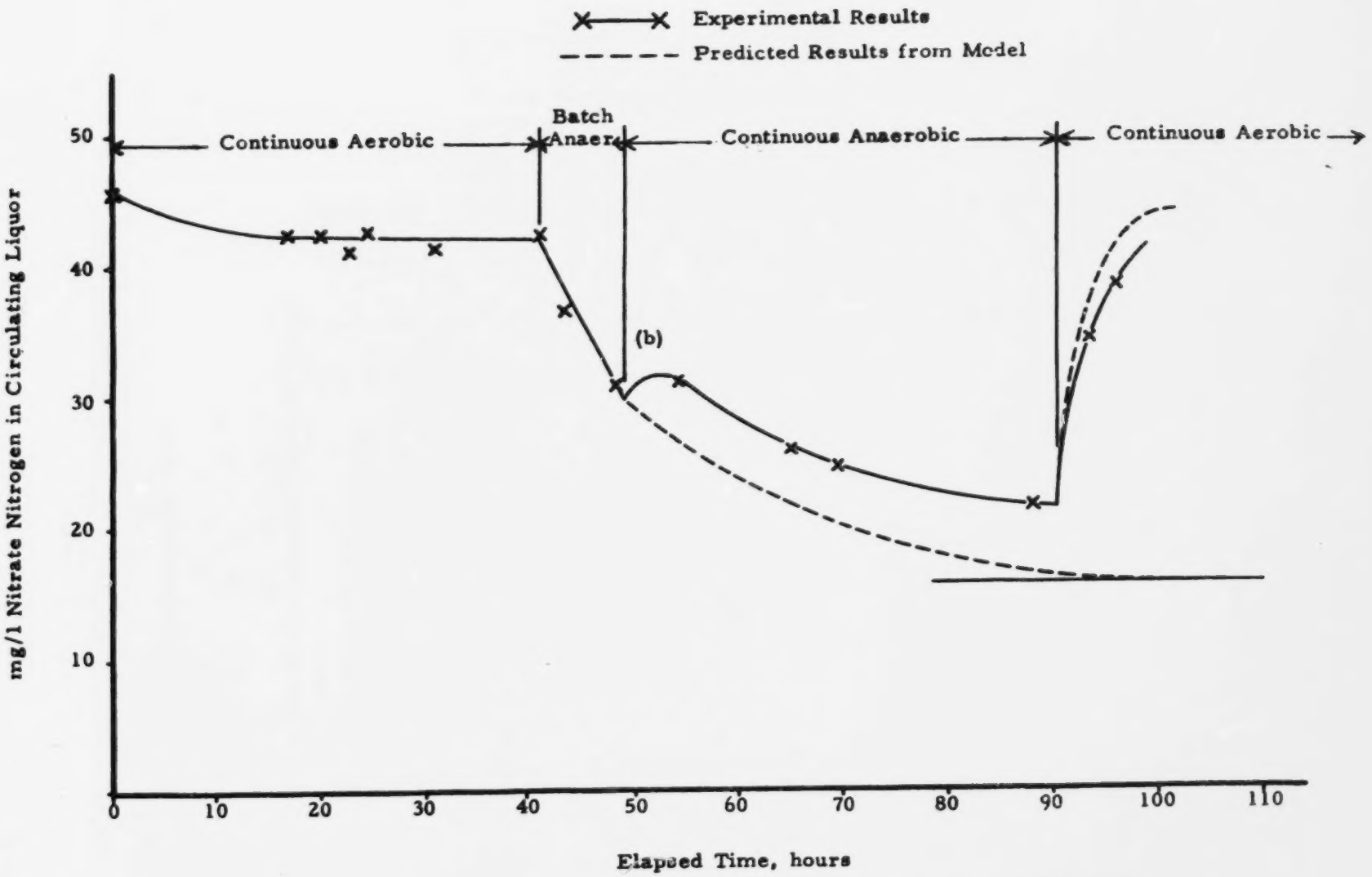


Figure 21. Aerobic-Anaerobic Cycling; a Comparison of Experimental and Predicted Results. Unit 1, Run 111769, 18°C. Unit switched from aerobic to anaerobic mode at 40.7 hrs. Brief batch phase preceded continuous operation to determine an initial denitrification rate for use in the model. This was later found unnecessary. Flow rate = 2.5 ml/min.

TABLE 8

CHANGES IN AMMONIA CONCENTRATION DURING CYCLED OPERATION

(Run 111769)

Hours	Elapsed Time From Phase Switch	Phase (Table 7)	NH_4-N (mg/l) Unit 1	Unit 2
0		a	2.2	1.4
16.8			0.4	7.9
20.0			1.1	8.7 ←
22.7	22.7		1.0	1.0
24.5	24.5		1.0	0.5
31.0	31.0		0.4	6.4
40.7	40.7		0.2	8.2
43.0		b	3.0	6.6
45.0			3.8	2.0
48.0			4.1 ←	1.5
54.0	13.2		1.0	0.8
65.0	24.2		0.5	0.7
69.5	28.7		0.9	0.7
73.5	32.7		2.4	4.1
88.0	47.2		3.0	5.6
90.0	3.0	c	1.0	4.5
93.0	5.5		1.1	5.5

Blocked-in figures enclose the anaerobic stage of each run.

Arrows denote the times the pumps were switched on to begin continuous operation.

effluent feed continues there is an apparent decrease in the rate at which nitrates are produced deviating from the amounts predicted by the model. A preliminary assumption might be that it is due to incomplete nitrification, but since ammonia and nitrite levels in the liquid at this time are negligible, this hypothesis can be rejected. A more likely explanation to account for the missing nitrate is to attribute it to nitrogen assimilation by the organisms of the biomass. Once restored to an aerobic environment, the cells begin to synthesize enzymes and replace depleted amino acid pools preparatory to resuming cell growth. Although the cells completely recover their oxidative abilities after anaerobiosis, the apparent decrease in nitrification is due to part of the incoming nitrogen being shunted into the cells' synthetic pathways.

The response of nitrifying beds to anaerobiosis can be qualitatively described: after shutting off air and primary effluent feed, the dissolved oxygen is depleted and utilization of recirculating nitrate begins. In batch operation, the nitrate is used up at an experimentally constant rate, until it is ultimately depleted. In continuous operation, nitrified feed is added at a steady rate and the nitrate concentration in the circulating liquor of the anaerobic unit gradually decreases to an equilibrium level which is a balance between the rate of nitrate input and the rate of nitrate utilization. The concentration of nitrate in the effluent of the unit equals that of the circulating liquor.

Since the feed to the anaerobic unit in continuous operation is the highly oxidized effluent from an aerobic system, the anaerobic microflora must rely on their stored reserve materials for a source of energy-yielding organic carbon. When these reserves approach exhaustion, rate of utilization of nitrate is reduced. Ultimately, under the stress of extended deprivation of available nutrients, the microflora undergoes major physiological changes that jeopardize its changes for survival when again placed in a favorable environment. Thus, it is essential that the aerobic microflora be returned to aerobiosis and a nutrient source of organic carbon before irreparable damage to its oxidative capability occurs. In cycled operation, therefore, it is important to know the progress of denitrification during the anaerobic phase. To enable the prediction of the equilibrium nitrate level, and to determine an optimum time for recycling, the mathematical model of our system was useful.

DENITRIFICATION RATE AS A FUNCTION OF TEMPERATURE

Tables 7 and 9 present continuous operating rate determinations, resulting from cycled aerobic-anaerobic operation at 18° and 29° C, respectively. The measurements of rate R during a given anaerobic cycle were obtained by solving the following equation, for R:

TABLE 9

CONTINUOUS RUN DENITRIFICATION RATES AT 29°C

Run No.	Phase	Elapsed Hours from Phase Switch	Rate (mg/hr / g MLVS) Unit 1	Unit 2
1570	a	7.5	0.578	
		26.0	0.523	
		50.0	0.632	
	b	5.0		0.705
		23.3		0.580
		48.0		0.532
	c	5.0	0.519	
		25.2	0.555	
		50.2	0.555	
		74.7	0.537	
		94.4	0.592	
	d	6.5		1.04
		24.2		0.797
		51.5		0.757
		75.2		0.755
	e	68.3	0.373	
		93.8	0.373	
	f	5.0		0.469
		23.8		0.584
		48.0		0.560
		75.0		0.596
	g	73.5	0.196	
		96.0	0.050	

TABLE 9 (Cont'd)

CONTINUOUS RUN DENITRIFICATION RATES
AT 29°C

Run No.	Phase	Elapsed Hours from Phase Switch	Rate (mg/hr / g MLVS) Unit 1	Unit 2
1570	h	19.5		0.693
		28.0		0.674
		72.2		0.586
	i	23.6	0.305	
		72.9	0.337	
		78.8	0.487	
		95.3	0.555	
	j	20.0		0.684
		28.5		0.687
		49.0		0.605
		73.0		0.663

$$R = \frac{FN_f - FY(t)}{1 - e^{-a(t-t_1)}} + \frac{F.a.\ N^{t_1}}{a-b} \cdot \frac{e^{-b(t-t_1)} - e^{-a(t-t_1)}}{1 - e^{-a(t-t_1)}} \qquad (5)$$

The results from these two tables plus the results of the batch experiments previously reported were combined and averaged for each unit at 18° and 29°C, and are presented in Table 10. Thus, each of the four rate values in Table 10 is the result of averaging approximately 20 independently obtained rates. The standard deviations, obtained by statistical analysis, indicate that the reliability of each value is high. The combined rates for both units presented as a function of temperature in Figure 22 show a 2.3-fold increase in denitrification for an 11°C temperature increase. This conforms remarkably well with the maxim of chemistry and biology that a 10° increase in reaction temperature can be expected to yield a doubling of reaction rate. Denitrification rate at 18° is 0.30 mg/hr per gram volatile solids, and at 29°C is 0.68 mg/hr per gram of volatile solids.

DISSOLVED OXYGEN UTILIZATION AND CHEMICAL CHANGES IN THE RECIRCULATING LIQUORS

Oxygen Level and Denitrification

During continuous cycled operation there is a continuous level of 7.5 8.0 mg/ℓ of dissolved oxygen (DO) entering the anaerobic unit, along with the nitrified feed. In order to evaluate the effects of this oxygen on the course of denitrification, a series of experiments was carried out to monitor the various parameters of interest.

Arrangements were made to include an oxygen probe in the system to measure this parameter until a steady state DO level was achieved. Ammonia, nitrite, and nitrate nitrogen levels were followed as well as the COD at the beginning and the end of the run. In order to detect subtle metabolic changes in the microflora during the course of the runs, phosphates, amino acids (as tyrosine), and protein content of the effluents were also followed.

Figure 23 presents the oxygen profile of two series of denitrification runs in which the DO of the incoming nitrified effluent was close to saturation at 8.0 ppm. These curves show the decline in the DO concentration of the recirculating liquor that was in the system at the time the aeration was stopped. In the later portion of the curve the slope is somewhat modified by the incoming DO of the influent feed and always seems to taper off to a minimal value around 0.5 mg/ℓ. The significance of this low base-line is not yet understood but possible later refinements in technique may permit restudying this effect.

Denitrification run 91169 was carried out at a residence time of 125 minutes. This meant that a relatively high input flow rate of 20 ml/minute nitrified effluent was required. The high flow was used to

TABLE 10

DENITRIFICATION RATES VS TEMPERATURE

Temperature, °C	Denitrification Rate* (mg/hr / MLVS) Unit 1	Unit 2
18	.29 ± .04	.31 ± .13
29	.70 ± .13	.66 ± .15

* Average includes both the batch and continuous denitrification runs.

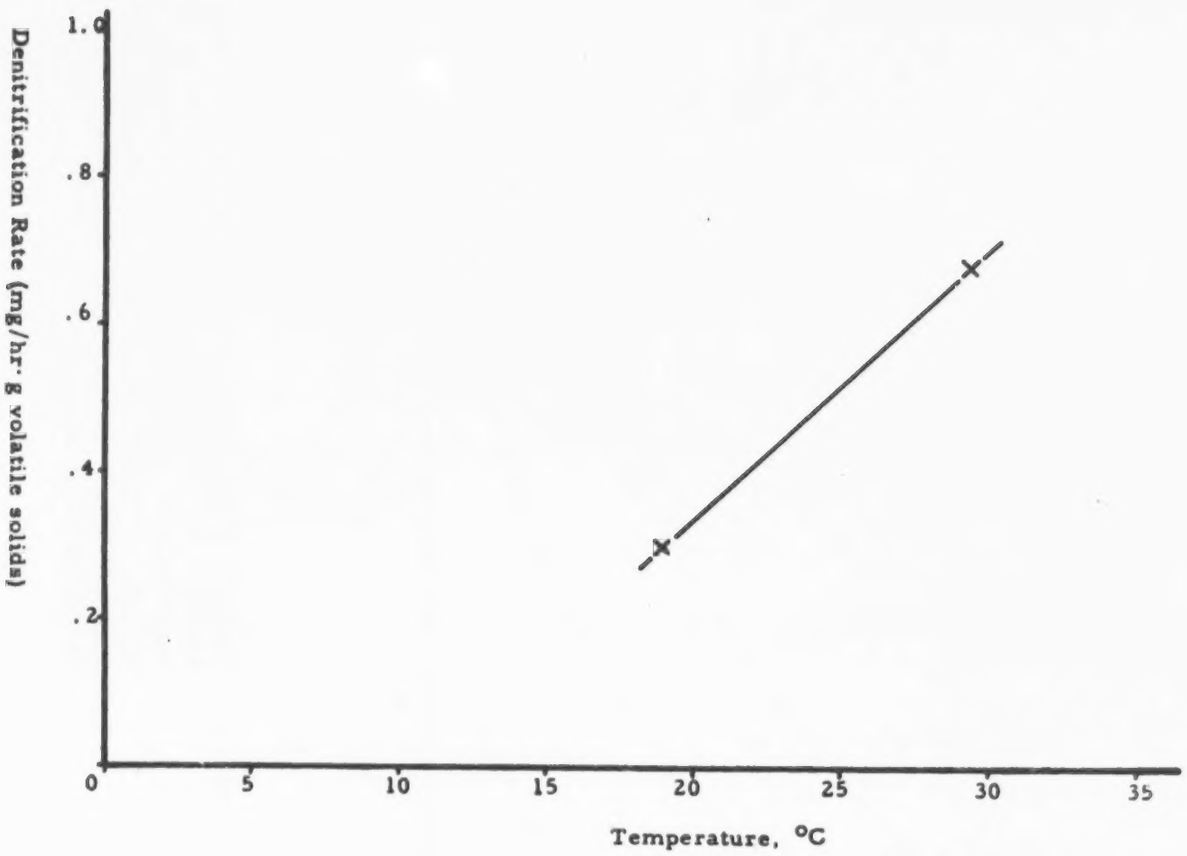


Figure 22. Influence of temperature on denitrification rate. All runs from both the batch and continuous experiments are included. The values for Unit 1 and Unit 2 were also averaged together.

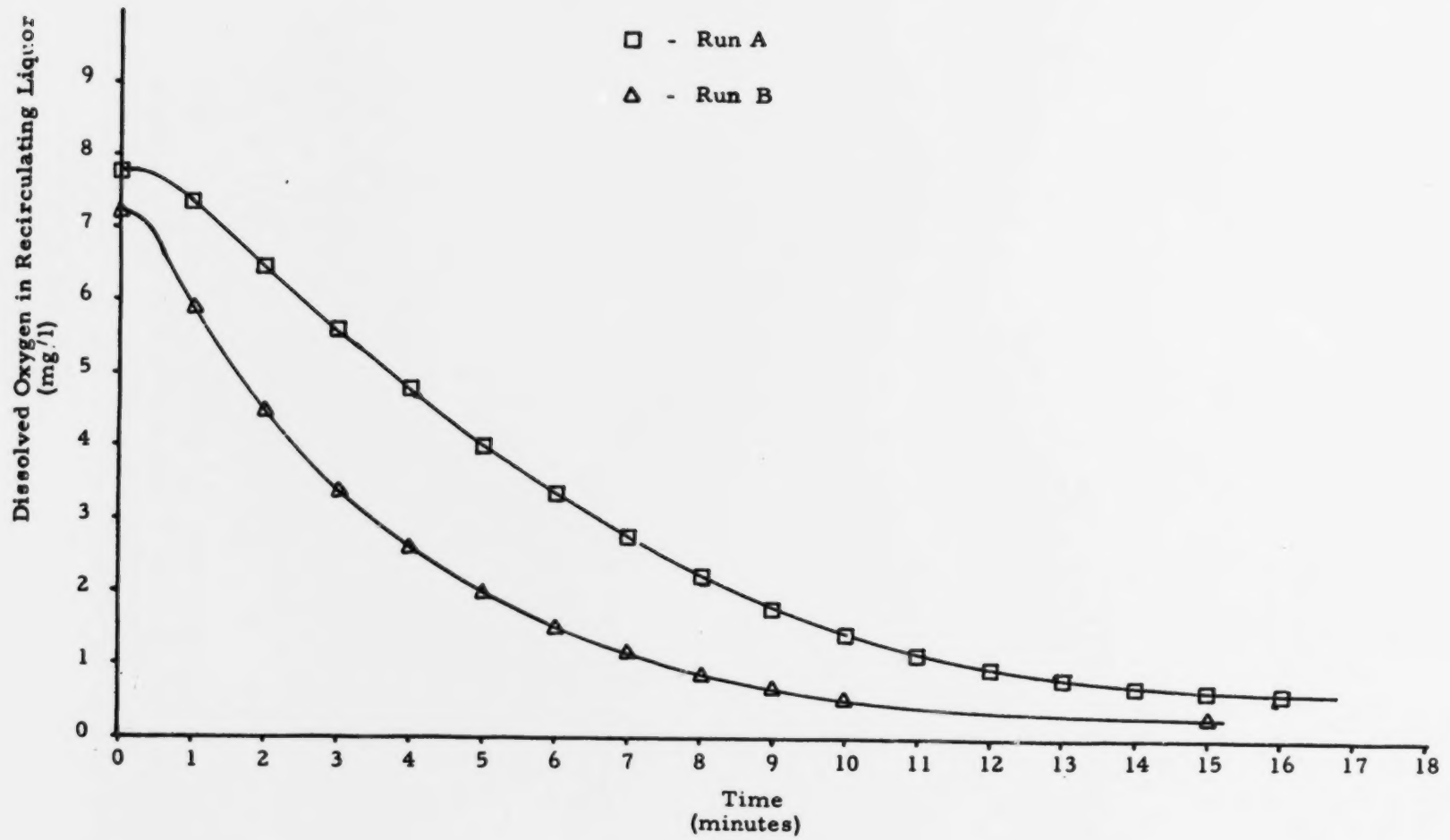


Figure 23. Oxygen utilization with units connected in series Run 91169 - 29°C. Unit was aerobic prior to start of the experiment. At t = 0, the air and sewage flows were turned off and the nitrified feed started at 20 ml/min. The 0.5 mg/l residual DO remained constant throughout the remainder of the run.

insure that a metabolically significant amount of oxygen would be entering the unit along with the feed. The amount of DO being made available to the microflora in the "anaerobic" bed during this run was calculated on the basis of a feed flow of 20 ml/min with a DO concentration of 8.0 mg/ℓ .

The rate of C_2 input is thus:

$$8.0 \text{ mg}/\ell \times 0.020 \text{ /min} = 0.16 \text{ mg } O_2/\text{min}$$

$$\frac{0.16 \text{ mg } O_2/\text{min}}{2.5\ell \text{ (volume of chamber)}} = \frac{0.064 \text{ mg } O_2}{\ell/\text{min}}$$

Assuming steady state conditions, this represents a Q_{O_2} of 0.43 1 O_2/mg MLVS / hr. This low Q_{O_2} is about 5% of the oxygen required for maintaining aerobic respiration and is comparable to the endogenous respiration rate of starved cells, in a Warburg vessel, that are metabolizing their carbon reserves. It is important to determine if during denitrification this low level of respiration makes up a significant proportion of the biomass metabolism thus affecting the rate of reduction of NO_3^-.

Analyses of the effluents in run 91169 during continuous denitrification showed that the nitrites in the incoming feed were almost completely removed (Figure 24) while the nitrate level decreased to its equilibrium point of about 2 mg/ℓ being removed. Overall nitrogen removal was about 10% of the total incoming nitrogen (Figure 25) which represents a denitrification rate of 0.75 mg/hr / g MLVS. This rate is in agreement with the values previously reported using residence times of 1000 minutes and achieving over 90% nitrogen removal. It may be concluded that an oxygen input of 0.43 $\mu\ell$ O_2/mg MLVS/hr has a negligible effect on the denitrification capacity of the microflora. Since to achieve a 90% NO_3^- to N_2 conversion the denitrification system is operated at about a 1000 minute residence times the oxygen made available to the biomass would be only about 0.005 $\mu\ell$ O_2 /mg MLVS/hr. This would be quickly consumed through respiration and would result in a dissolved oxygen concentration close to zero in the recirculating liquor.

A continuous denitrification experiment wherein the residence was doubled over the run presented in Figures 24 and 25 is illustrated in Run 10269, Figure 26. The nitrite-nitrate values presented in this figure have been corrected for the dilution of the incoming feed with that already present in the unit at the start of the run. At 10 ml/min it takes approximately 10 hrs to replace 90% of the liquid originally present in the unit.

Once steady state is achieved, 40 mg/l NO_3-N is entering the unit and 26.5 mg/l NO_3-N is present in the effluent. This means that 13.5 mg/l or about 34% of the incoming NO_3-N is being removed.

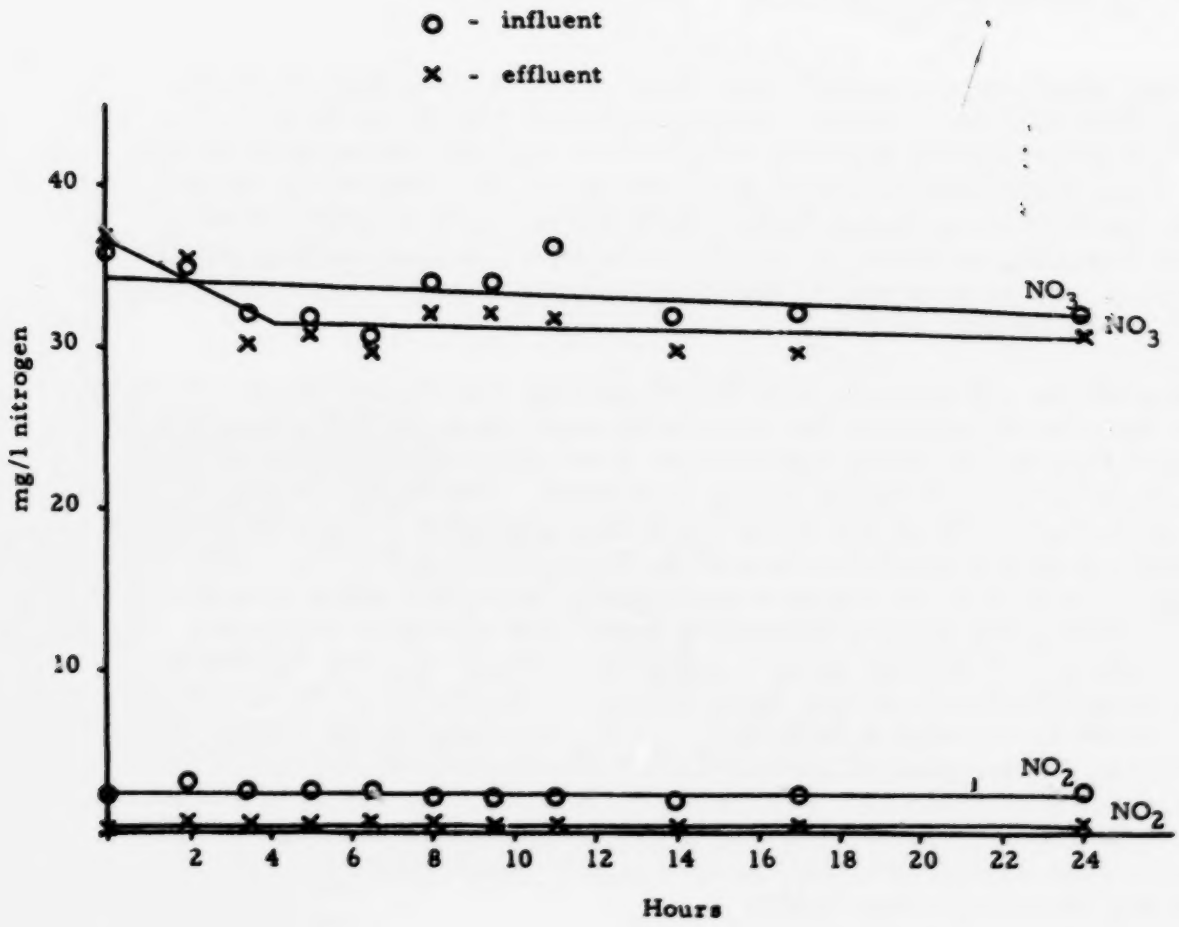


Figure 24. Continuous denitrification with an oxygen saturated feed. Run 91169, 29°C nitrified feed flow - 20 ml/min (residence time - 125 min). Dissolved oxygen content of feed 7.8 mg/*l*. R = 0.75 mg/hr/g MLVS.

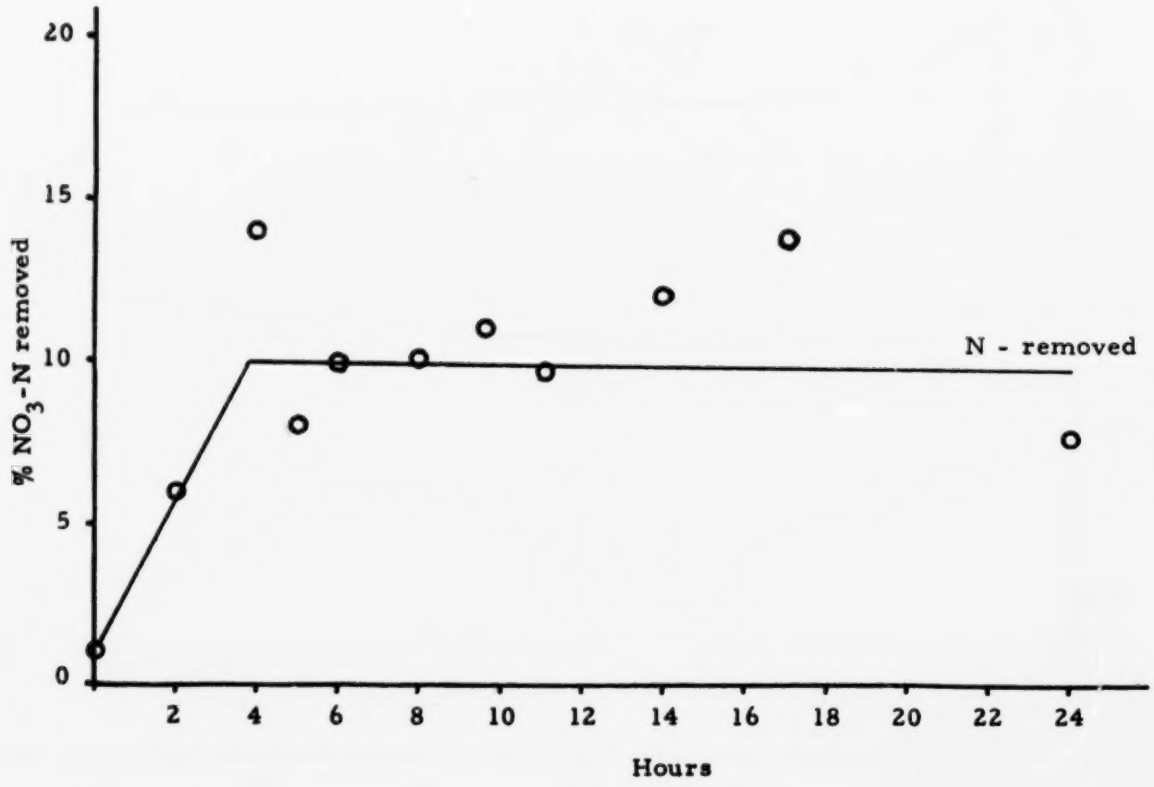


Figure 25. Fraction of total NO_3-N removed using an oxygen containing feed Run 91169, 29°C. Continuous denitrification with an oxygen saturated feed. Run 91169, 29°C, nitrified feed flow = 20 ml/min (residence time = 125 min). Dissolved oxygen content of feed = 7.8 mg/l. R = 0.75 mg/hr/g MLVS.

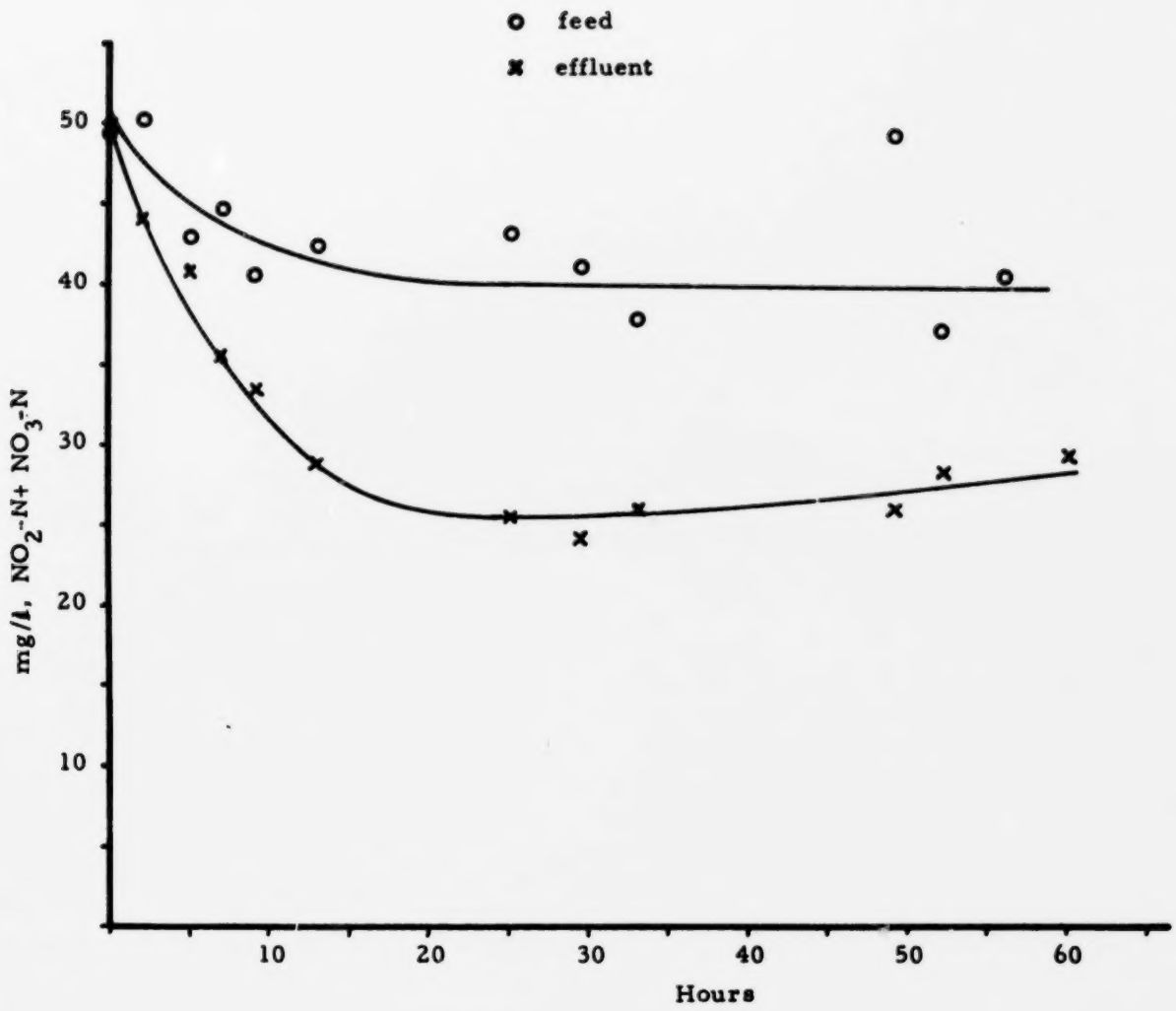


Figure 26. Continuous denitrification at increased residence time. Unit 2, Run 10269, 29°C. Flow rate of nitrified feed set at 10 ml/min (residence time = 250 min). R = 0.455 mg/hr/g MLVS.

At 10 ml/min the residence time in the system is 250 minutes or 4.16 hrs. Then the rate of denitrification (R) is:

$$R = \frac{13.5 \text{ mg/l}}{4.16 \text{ hrs} \cdot 7.1 \text{ g MLVS}} = 0.455 \text{ mg/hr / g MLVS}$$

This rate compares rather favorably with the values for the batch studies already presented.

The significance of these studies is yet to be fully developed. There obviously is an upper limit to the amount of DO that can be tolerated in a denitrification unit. What this maximum permissible level is and whether denitrification is an all or none affair, that is, can O_2 and NO_3^- be utilized simultaneously, will have to be answered in later studies.

Proteins, Amino Acids and Nitrogen in Feeds and Effluents

The chemical analysis of the runs used for the oxygen studies is presented in Figure 27. There was very little change in the $PO_4^=$, amino acids and total protein in the effluent of the anaerobic unit as compared to the influent indicating that the integrity of the microflora has not been altered by test conditions - at least for the first 24 hours.

The presence of amino acids and protein in the supernatant liquor from the various treatment units also offers some clues as to the physiological state of the biomass. Accordingly, the data in Table 11 was collected on a series of continuous runs up to 90 hours in duration. There were no apparent trends with time from the start of a phase switch and so the data presented in the table are averaged for the entire series and presented in Table 12.

The incoming primary effluent, as might be expected, contains material that reacts both with the Lowry protein analysis and the colorimetric test for tyrosine. These materials are reduced by about 58% during aerobic treatment and the amount of tyrosine material changes by an additional 10% during the anaerobic phase. The protein like material is reduced by another 60% during anaerobiosis giving an overall removal of about 80%.

The significance of these observations is not clear. There are a number of phenolic compounds and reducing agents that can interfere with both of these tests. A more definitive resolution of the reactants perhaps through chromatography is needed before they can be accepted as proteins and amino acids.

The reactants appear to be materials that enter with the primary effluents, portions of which are refractory to biological attack. The

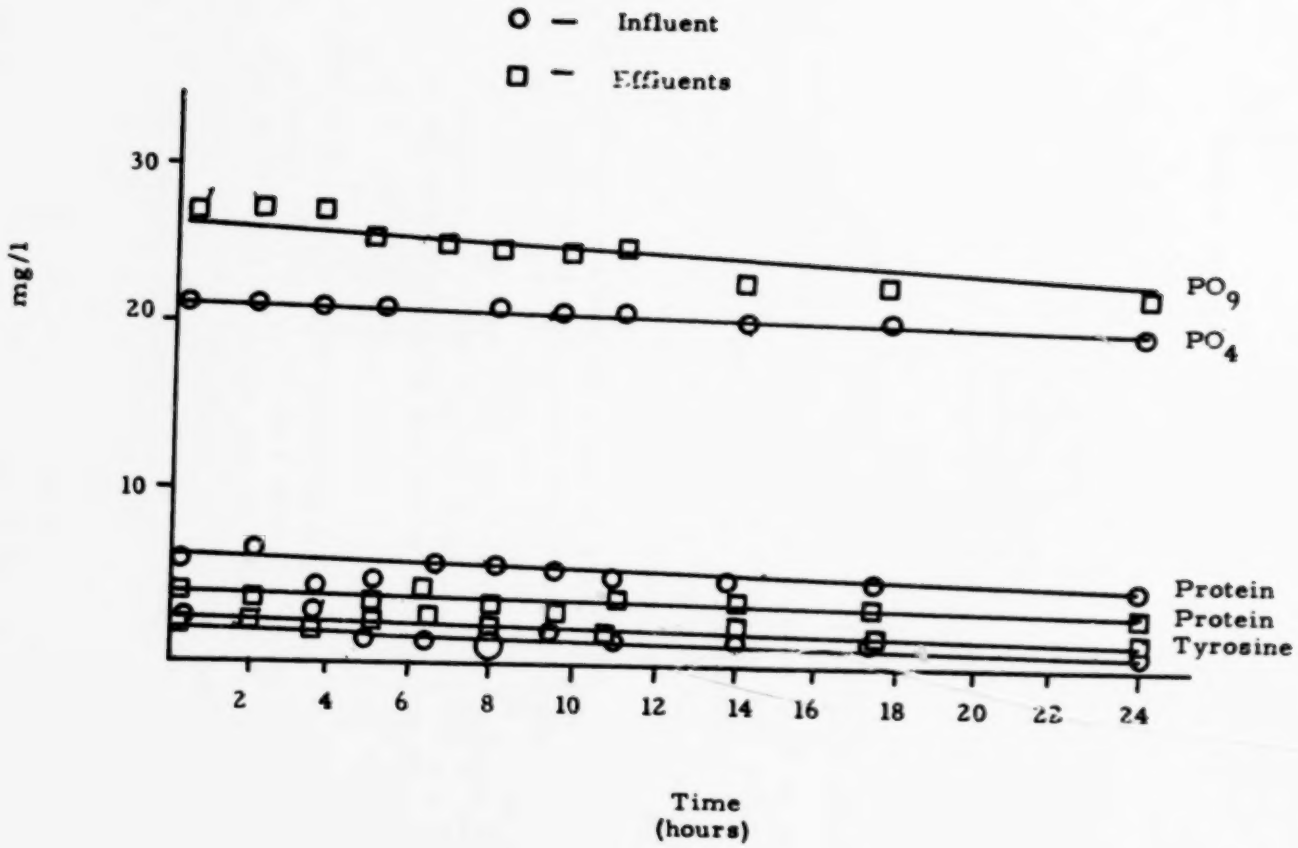


Figure 27. Chemical changes between influent and effluent flows during denitrification, Run 91169, 29°C. Flow rate 20 ml/min. Phosphates were determined using procedures described in standard methods; proteins were determined by the Lowery procedure and the method of Spies was used for the tyrosine analysis (see Appendix I).

TABLE 11

PROTEIN AND AMINO ACID CONTENTS OF TREATMENT SYSTEM EFFLUENTS - RUN 1570

Elapsed Time	Hours from Phase Switch	Phase	Mode	Proteins mg/ℓ	Tyrosine mg/ℓ	Total Amino Acids (Tyrosine x 20) mg/ℓ
			UNIT 1			
529	Azusa feed			25.0	4.5	90
529	0	f	AER	8.5	2.0	40
534	5			10.5	1.7	35
553	24			8.5	1.8	37
577	48			9.0	2.5	50
604	75			8.0	1.7	35
604	Azusa feed			15.5	5.0	100
			UNIT 2			
529	0		AN	8.5	1.9	37
534	5			7.0	2.0	40
553	24			6.5	2.0	40
577	48			4.5	2.2	45
604	75			10.5	2.0	40
			UNIT 1			
700	Azusa feed			24.0	10.2	204
700	0	h	AER	-	3.0	60
720	20			7.0	2.7	55
728	28			4.5	2.7	55
773	73			-	5.5	110
773	Azusa feed			8.0	12.2	245
			UNIT 2			
700	0		AN	9.5	2.7	55
720	20			4.5	3.0	60
728	28			2.0	3.0	60
773	73			-	3.5	70

TABLE 12

SUMMARY

PROTEIN AND AMINO ACID CONTENTS OF TREATMENT SYSTEM FEEDS AND EFFLUENTS - RUN 1570*

	Proteins mg/ℓ	Tyrosine mg/ℓ	Total Amino Acid (Tyrosine x 20) mg/ℓ
Primary Effluent	20.00	8.80	176.0
Aerobic Effluent	6.65	2.85	57.0
Anaerobic Effluent	3.95	2.63	52.6

Flow rate into aerobic unit = 30 ml/min

Flow rate into anaerobic unit = 4.0 ml/min

Ave MLVS = 6.3 g/ℓ

*Includes phases f, h, i, and j (529-604 and 700-942 hours of continuous coupled operation).

small difference between the aerobic and anaerobic effluents shows that both environments operate as metabolically aerobic systems which would be the case if NO_3^- was the sole electron acceptor. Even if the Lowery reactive materials were released as leakage products by the sludge mass itself, they would represent only a very small portion ($\sim 0.2\%$) of the proteins contained in the biomass.

INFLUENCE OF EXTERNAL COD ON DENITRIFICATION RATES

Measurements of COD levels in the influent and effluent of the activated bed units during an anaerobic phase of cycled operation showed that oxidation of some of the residual COD in the nitrified effluent occurs during denitrification. Figures 28 and 29 illustrate this in run 1570 during continuous operation. As a method for approximating the rate of COD oxidation during anaerobiosis, as for example in the phase depicted in Figure 28, we can use the fact that after 30 hours a stable COD level in the effluent was achieved. This represents an equilibrium balance between a constant rate of oxidation and a constant input of nitrified influent. The amount of COD oxidized in this case was 16 mg/l, and flow through the system was 5 ml/min. Thus, the removal rate was:

$$\frac{16 \text{ mg}}{\text{L}} \times \frac{5 \times 10^{-3} \text{L}}{\text{min}} \times \frac{60 \text{ min}}{\text{hr}} \times \frac{1}{17.8 \text{ g MLVS}} = 0.269 \frac{\text{mg COD}}{\text{hr} \cdot \text{g MLVS}}$$

This rate of actual exogenous COD oxidation during anaerobiosis is meaningful when compared to the potential for such oxidation provided by nitrate reduction during denitrification. Average denitrification rate for anaerobic phase d, Run 1570 above was 0.755 mg NO_3-N/hr / g MLVS. To convert this value to oxidative potential we need to consider a balanced chemical equation describing denitrification.

A commonly used overall chemical description of denitrification is:

$$5\ CH_2O + 4\ NO_3^- \longrightarrow 2\ N_2 + 5\ CO_2 + 3\ H_2O + 4\ OH^-$$

An equivalent equation describing a similar oxidation by oxygen is:

$$5\ CH_2O + 5\ O_2 \longrightarrow 5\ CO_2 + 5\ H_2O$$

Clearly, the capacity of 4 moles of NO_3^- to oxidize 5 moles of CH_2O is equivalent to the capacity of 5 moles of O_2. In other words, 1.00 mg of nitrate expressed as nitrate nitrogen is the equivalent of 2.85 mg of O_2 in terms of oxidizing capacity in our system.

To properly compare the potential to oxidize organic carbon with actual oxidation of exogenous substrate we also need to consider the state of metabolic activity of the microflora. A treatment unit is essentially a continuous culture device with influent nutrient flow rate adjusted to give minimum cell yield (wastage of produced cell material in the effluent) and maximum oxidation of influent organic materials. The culture as a whole resides in the late logarithmic

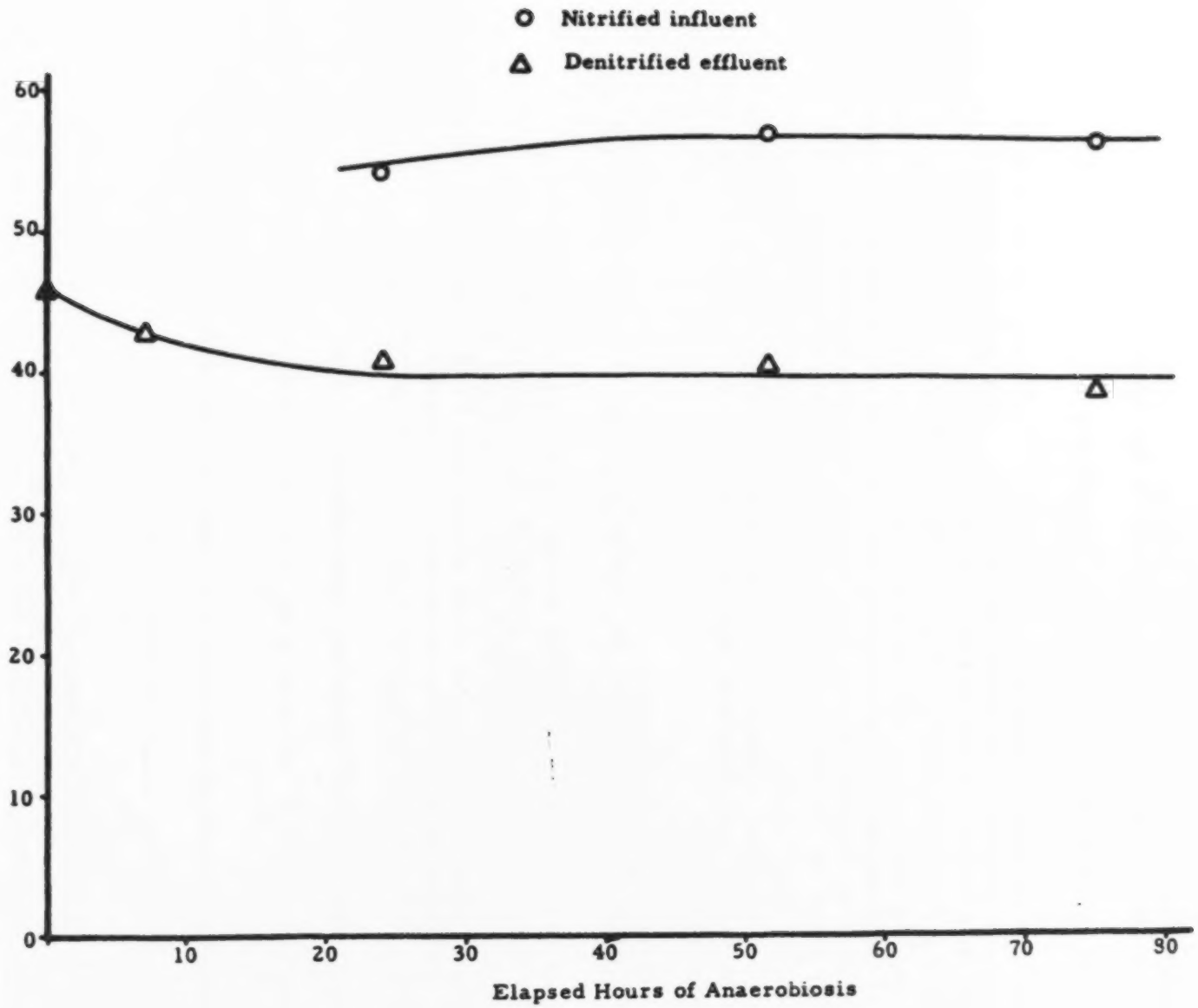


Figure 28. Oxidation of residual COD entering the denitrification unit. Unit 2, Run 1570, phase d, 29° C. Flow of nitrified feed = ml/min (residence time = 500 min). Rate of removal of COD R = 0.269 mg/hr/g MLVS.

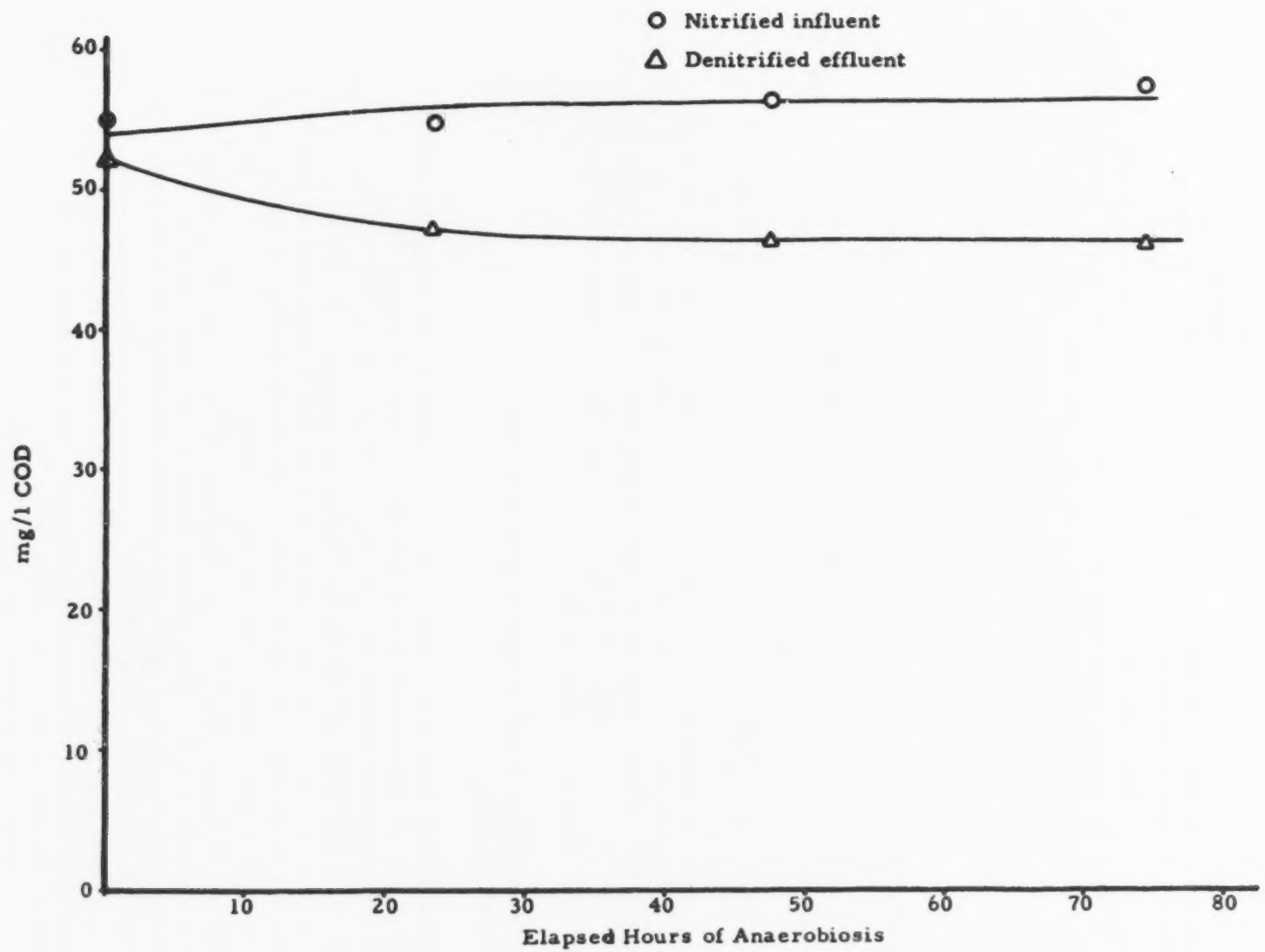


Figure 29. Oxidation of residual COD entering the denitrification unit. Unit 1, Run 1570, phase f, 29°C. Oxidation of residual COD entering the denitrification unit. Unit 2, Run 1570, phase d, 29°C. Flow of nitrified feed = 5 ml/min (residence time = 500 min). Rate of removal of COD R = 0.269 mg/hr/g MLVS.

phase of the typical microbial growth curve. In this condition, the flora, while still viable and exhibiting a small growth rate, uses most of the available organic carbon in the external medium for maintenance energy source. In the Activated Bed system, in other words, most of the influent organic carbon "removed" by the microflora is oxidized to CO_2, with a very small fraction being converted to excess cell material. Therefore, we can assume for purposes of discussion, a simple stoichiometric balance between carbon removal and oxygen consumption during aerobiosis, or between carbon oxidation and nitrate reduction during anaerobiosis, without the complication of the correction used by McCarty, Beck, and Amant (1969) for synthesis of cell material.

The denitrification rate of 0.755 mg NO_3-N/hr / g MLVS, therefore, represents a COD oxidation rate of 2.2 mg O_2/hr / g MLVS. Actual oxidation of exogenous COD was 0.269 mg O_2/hr / g MLVS, hence external COD accounted for 12.0% of the total oxidation which must have occurred in this denitrification phase. We can reasonably conclude that the remaining oxidation which must have taken place during denitrification was performed on internal substrates, i.e., endogenous reserves. Table 13 presents the results of similar calculations performed on data obtained from earlier runs, verifying the consistency of this behavior. This confirms that endogenous reserves are the predominant source of carbon utilized during equilibrium anaerobic operation of the Activated Bed units.

THE EFFECT OF LENGTH OF ANAEROBIOSIS ON DENITRIFICATION RATE

As in the previous batch studies, rate - R - appears to be roughly constant for the duration of each anaerobic phase. This is especially true after about the first 10 hours of operation when the units settle down with minor fluctuations about some baseline level. In Table 9, denitrification rate results of cycled operation at 29° C are presented. Similarly, Table 7 presented the 18° C data. Relative stability in R can be seen in samples taken after 10 hours of anaerobiosis. The 10 hours again reflect the time required to replace 90% of the NO_3 containing liquor present at t = 0. The above data on utilization and availability of exogenous and endogenous carbon correspond with the hypothesized substrate effect. The stable low availability of biologically usable exogenous carbon seen after about 10 hours of anaerobic operation (Figures 28 and 29), forces the microflora to rely on endogenous carbon reserves. The fact that denitrification rate apparently remains stable after 10 hours in these runs implies that the rate of mobilization of endogenous reserves is constant. The reserves appear to be sufficient to furnish substrate for up to 75 hours without being exhausted.

TABLE 13

OXIDATION OF ENDOGENOUS AND EXOGENOUS CARBON SOURCES DURING DENITRIFICATION
(all rates are per hr/g MLVS)

Run No.	Unit	Denitrification Rate (mg NO_3-N)	Total Oxidation Rate (as mg O_2)	Actual Oxidation Rate of Exogenous COD (mg O_2)	$\frac{\text{Oxidation of Exogenous Carbon}}{\text{Total Oxidation}} \times 100$ (%)
10269	2	0.455	1.61	0.162	10.0
10769	2	0.490	1.40	0.214	15.3
102069	1	0.830	2.37	0.180	7.6
102069	2	0.762	2.18	0.167	7.7
1570d	2	0.755	2.15	0.269	12.0
1570f	2	0.580	1.65	0.218	13.0

Although both the nitrification and the denitrification phase take place in an aerobic environment, the denitrification rate is the slower of the two (5.2 mg/hr / g MLVS vs 0.67 mg/hr / g MLVS at 29°C). This apparent slowing of the metabolism of the microflora under anaerobic conditions may be an effect of substrate concentration. Typically, in metabolism and enzyme chemistry, as substrate concentration is reduced below a threshold level, reaction rate becomes substrate dependent. The critical nutrient limiting denitrification rate in this case may be internally or externally available carbon, or it may be the decreasing concentration of nitrate nitrogen itself. This will be discussed further in a later part.

Nitrification proceeds from 5-8 times faster than denitrification in these studies. If all of the effluents from a wastewater treatment plant are to be denitrified, some provisions for balancing the resultant difference in hydraulic flows will have to be made. This would require that the volume of the anaerobic tanks be 5-8 times the volume of the aerobic tanks. Such a system could be developed using a series of tanks wherein 1/5-1/8 of the tankage volume would be switched between aerobic and anaerobic modes at the appropriate time intervals.

Return of Denitrifying Capacity After Removal of the Biomass

The Activated Bed units were stripped down for an analysis of the biomass on 12/18/69 as indicated in Table 4. The units were reassembled with a small fraction of the microflora still clinging to the rings. They were then operated aerobically for a period of 2 weeks at a 70 min residence time prior to the start of run 1570.

The rate data for run 1570 presented in Table 9 shows that there did not occur any significant rate change through the entire series of cycles - phase a through j. This indicates that the units had been restored to their full denitrifying potential within two weeks after start-up.

Distribution of Nitrogen Compounds During Denitrification

As previously reported, there occurs a continuous low-level release of NH_4^+ into the surrounding liquid during denitrification.

A statistical analysis of NH_3 production for all previous batch anaerobic runs was completed and the rate of NH_3 appearance during anaerobiosis was estimated. For periods of anaerobic operation of less than 50 hours duration, the ammonia is produced at approximately a constant rate. There is some indication, however, that the ammonia production rate drops significantly after about 50 hours. The batch run experimental data gives a rate of $0.15 \pm .04$ mg/hr / g MLVS. Similar determinations carried out on continuously operating units calculated to the identical rate of 0.15 mg/hr / g MLVS at 29°C for anaerobic phases less than 50 hours in duration. Figure 30 presents the NH_3-N concentration data on a typical 29°C anaerobic

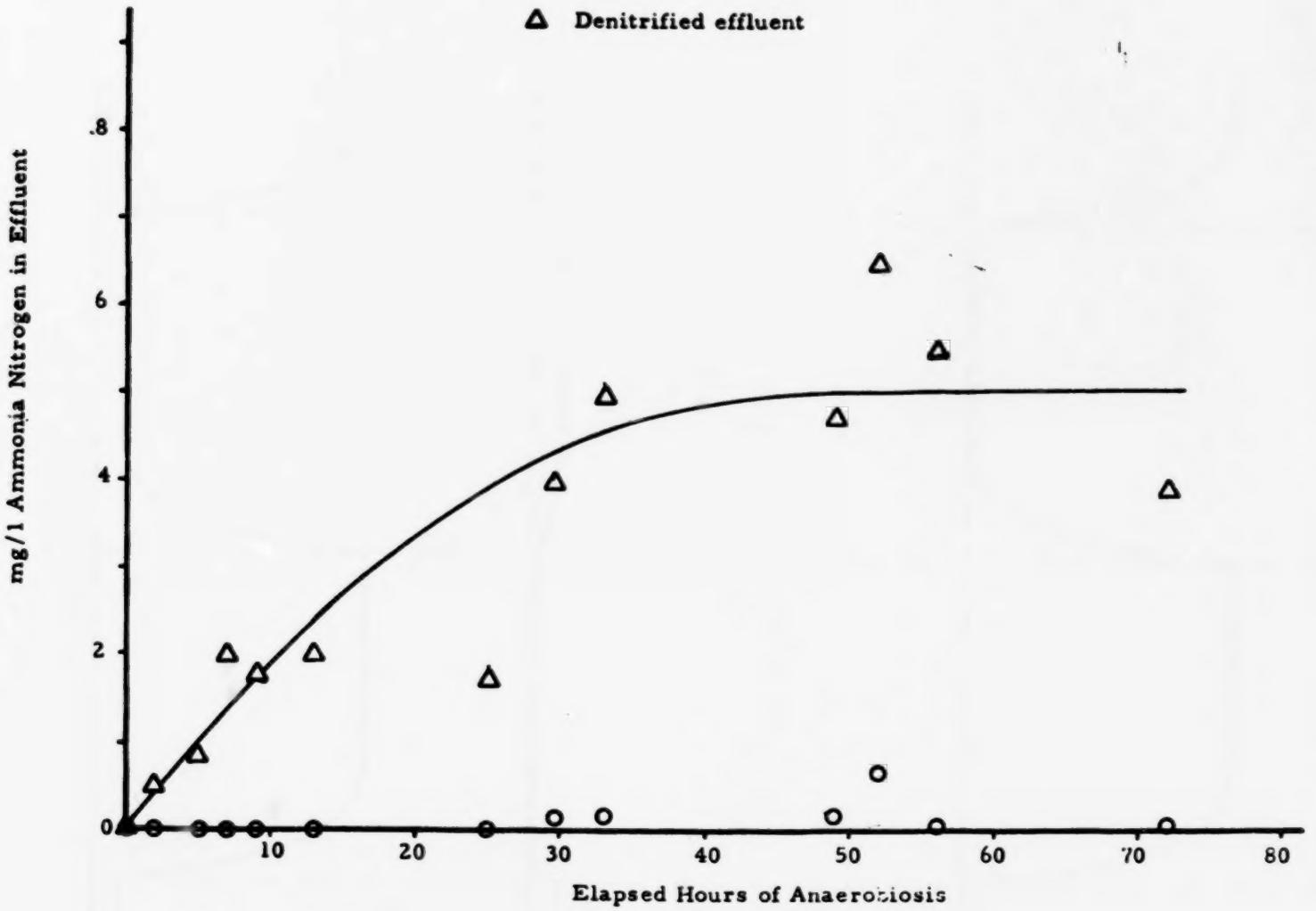
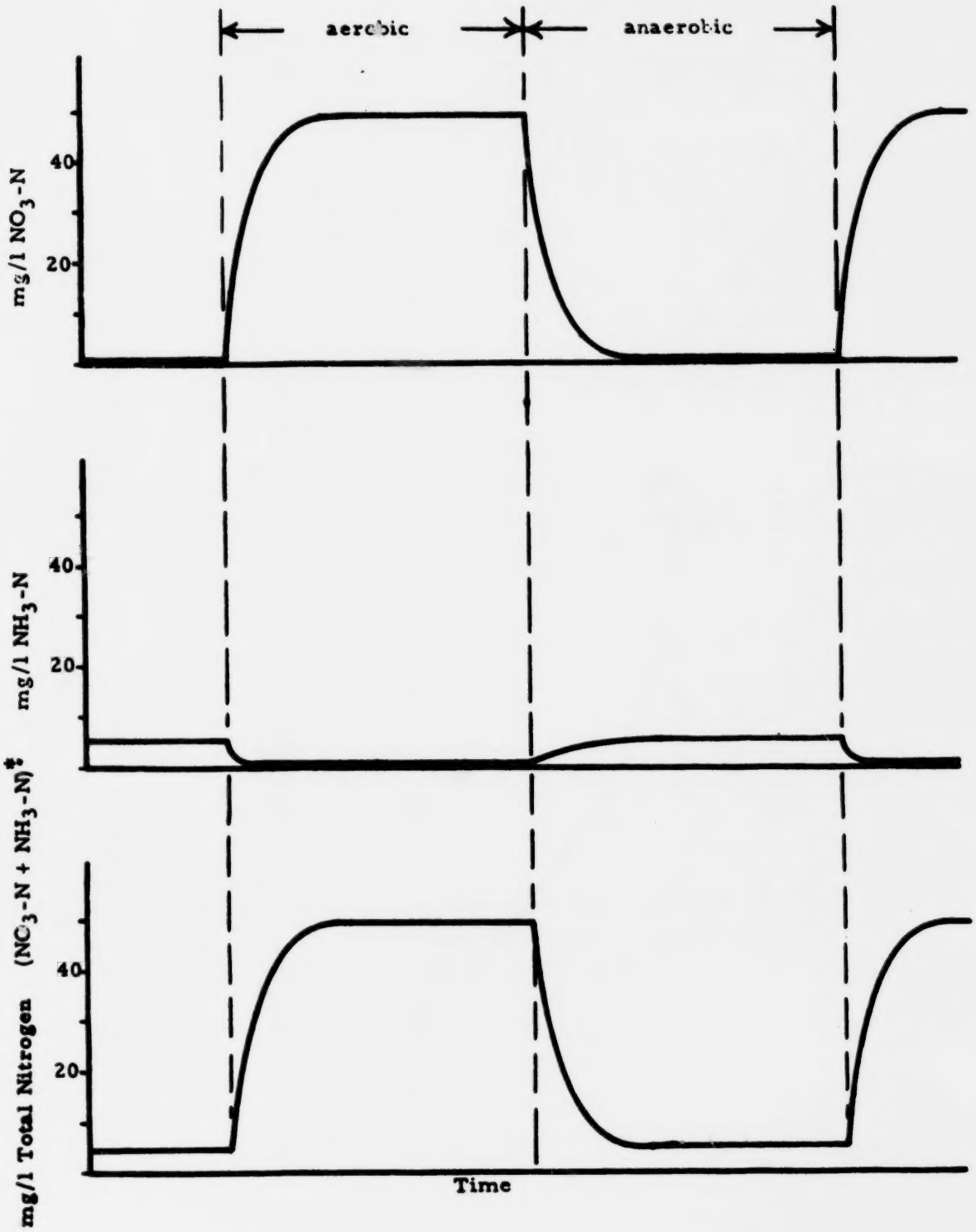


Figure 30. Buildup of ammonia during continuous denitrification. Unit 2, Run 10269, 29°C. Ammonia nitrogen is presumed to be released by the cell biomass rather than through the reduction of nitrate.



*Nitrite and organic nitrogen changes are insignificant at this scale.

Figure 31. Graphical illustration of the nitrogen transformations that take place in a single Activated Bed unit. The unit is being cycled between aerobic and anaerobic modes on the hypothetical time scale.

run. At 18°C, the rate of NH_3 production is 0.07 + 0.02 mg/hr / g MLVS. A linear function of temperature fitted to these points gives:

$$R_{NH_3}(T) = 0.062 + 0.0073T = \text{rate of } NH_3\text{-N appearance}$$

This is the factor incorporated into the model.

Nitrite nitrogen was found to be of little significance throughout most of the cycled operation of the units. In continuous operation of the Activated Bed units with stable microbial ecology, i.e., in units which have equilibrated to cycled operation, nitrite concentration are never greater than 1 mg/l NO_2-N at any time in either aerobic or anaerobic operation. Thus, nitrite nitrogen was not considered in the model.

Figure 31 shows graphically the inorganic nitrogen distribution during aerobic-anaerobic cycled operation.

Effects of Prolonged Starvation

Contrasted to the pattern of return to aerobiosis by relatively healthy beds, we see a different sort of behavior in Figure 32. In this case, Unit 1 had been subjected to continuous anaerobic operation for 162.5 hours, or approximately a week. Based on earlier data on the anaerobic performance of the bed, the level of nitrate nitrogen in the liquor at time 0 in Figure 32 should have been about 19 mg/1. The fact that about 33 mg/1 nitrate nitrogen were present indicates that a change in the rate of denitrification, R, must have occurred; i.e., the bed had lost much of its initial capacity to denitrify.

At time 0 in Figure 32 inputs of air and sewage were reinstated to the starved bed in Unit 1. The concentration of ammonia in the liquor immediately begins to rise, and the residual nitrate nitrogen is washed out of the system by simple dilution. Gradually the unit recovers, oxidation of ammonia increases in rate until it exceeds the rate at which ammonia is entering the system, ammonia level falls, and nitrate builds up. The nitrogen balance of the data presented in Figure 32 reveals ammonia, nitrate, and a trace of nitrite nitrogen introduced by the primary effluent feed. Very little nitrogen is diverted to the synthesis of protein or cellular material, indicating a lack of vitality in the bed, or a lagging response to a more favorable environment.

Subjected to extended anaerobiosis, then, the starved aerobic flora loses not only its denitrifying capacity, but also the ability to rapidly revert to vigorous aerobic oxidation. A graphical illustration of this loss of aerobic recovery capacity is presented in Figures 33, 34, and 35. Figure 33 shows as broken lines the hypothetical behavior of nitrate level in the circulating liquor if all influent ammonia is immediately converted to nitrate, Case 1, and if none of the influent

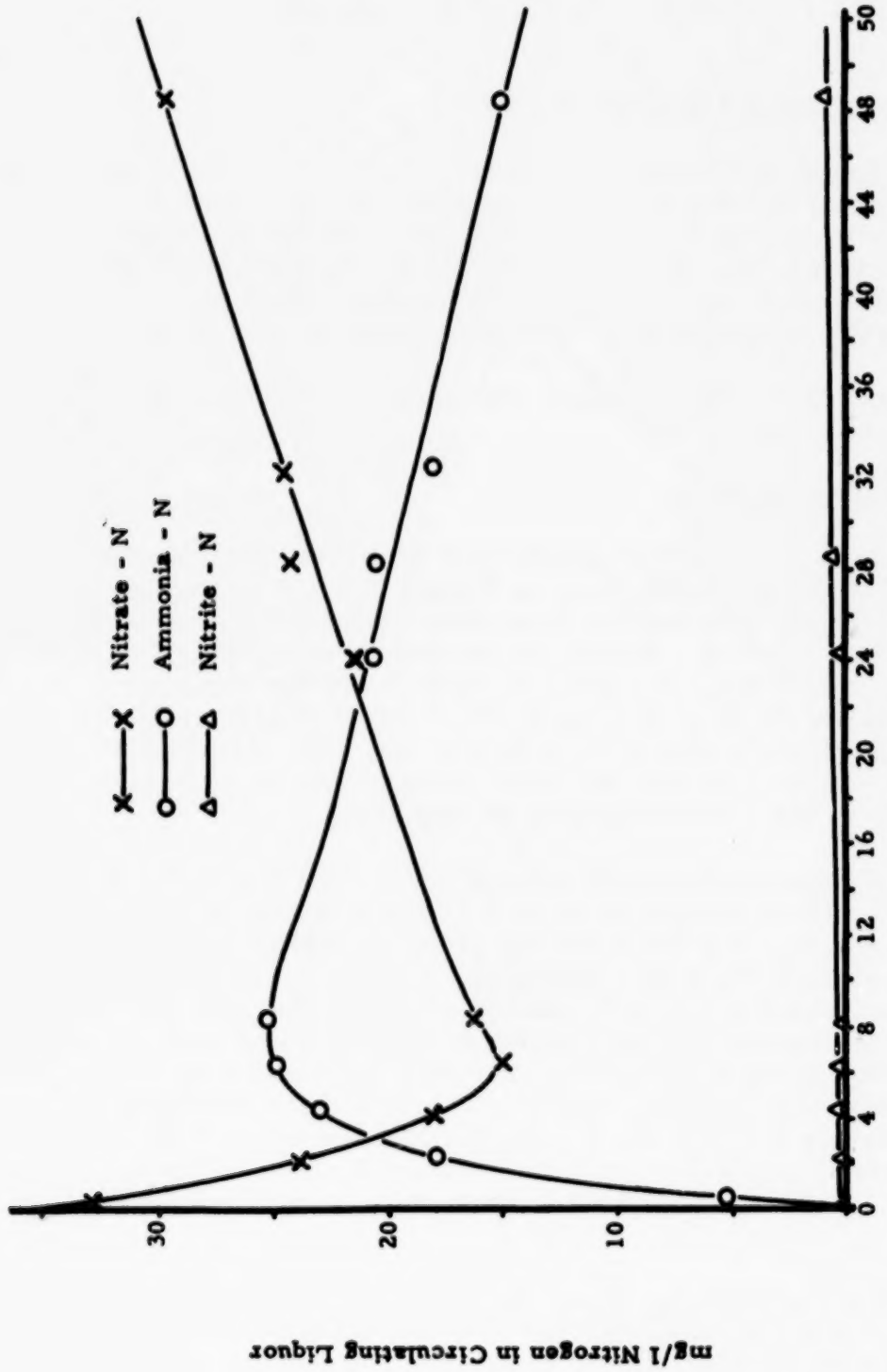


Figure 32. Effects of prolonged anaerobiosis on recovery of nitrifying capacity. Unit 1, Run 11769 - 29°C. Unit had been in the continuous denitrification mode for 162.5 hours before being returned to aerobiosis and primary effluent feed.

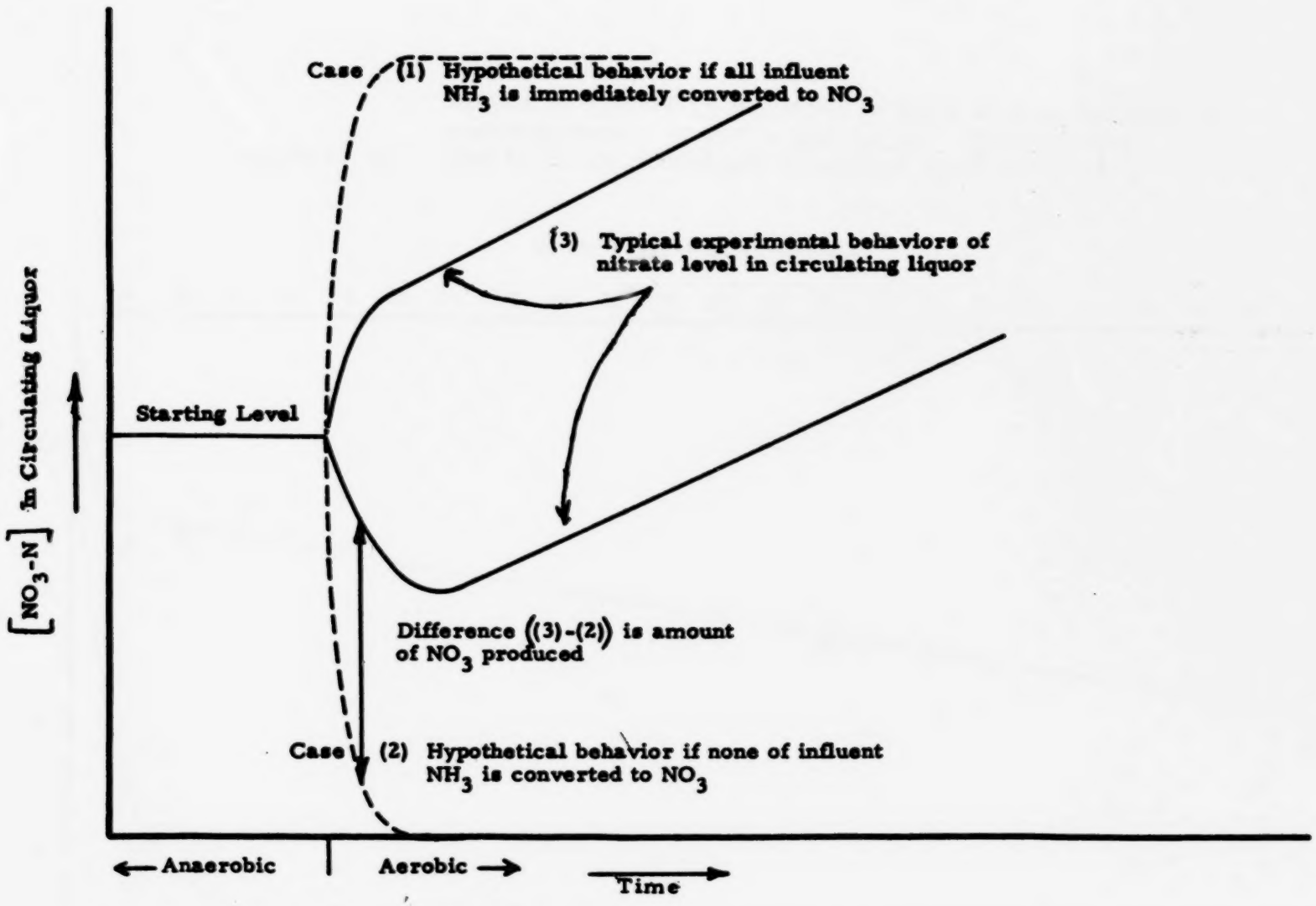

Case (1) Hypothetical behavior if all influent NH_3 is immediately converted to NO_3
(3) Typical experimental behaviors of nitrate level in circulating liquor
Starting Level
$[NO_3\text{-}N]$ in Circulating Liquor
Difference ((3)-(2)) is amount of NO_3 produced
Case (2) Hypothetical behavior if none of influent NH_3 is converted to NO_3
← Anaerobic
Aerobic →
Time

Figure 33. Graphical representation of possible physiological consequences of anaerobiosis. Expected behavior when the unit is returned to the aerobic mode.

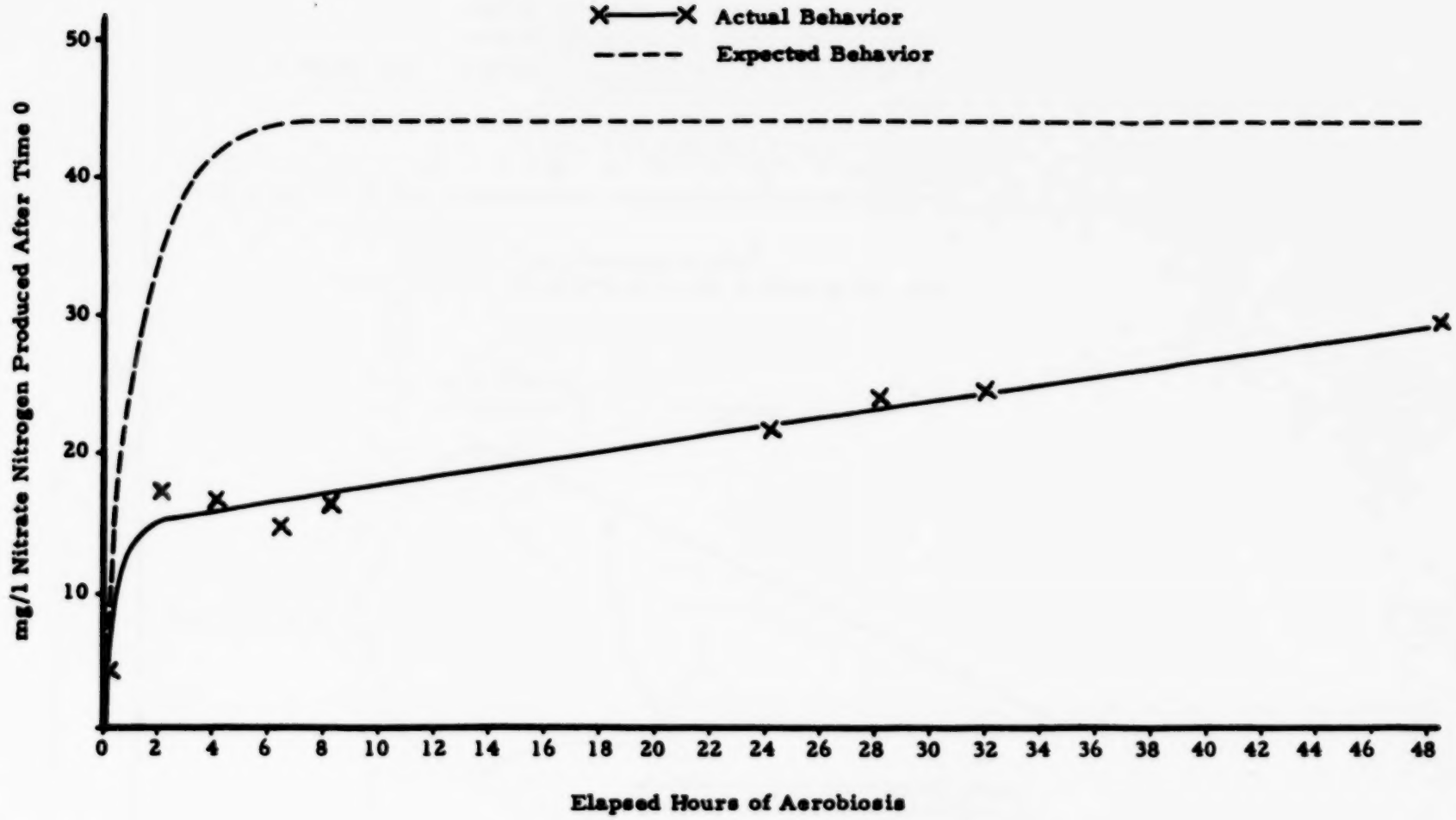

Actual Behavior
Expected Behavior
mg/l Nitrate Nitrogen Produced After Time 0
50
40
30
20
10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
Elapsed Hours of Aerobiosis

Figure 34. Recovery of nitrifying capability after prolonged anaerobiosis. Unit 1 - Run 11769. Experiment shown in Figure 32 adjusted to show nitrate production.

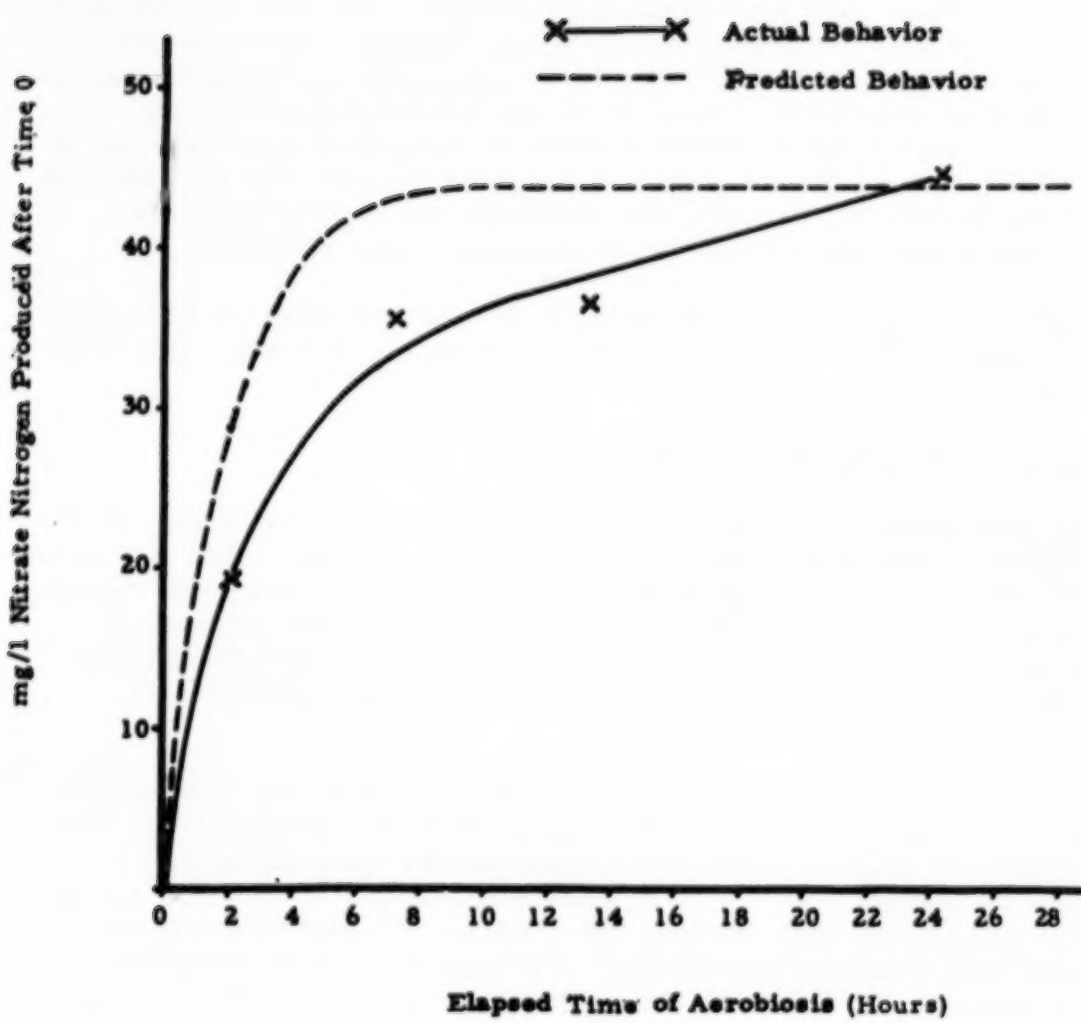


Figure 35. Recovery of nitrifying capability after anaerobiosis. Unit 2 - Run 11769; 29°C.

ammonia is convertedto nitrate, Case 2, the nitrate decreasing by dilution with the incoming feed. Actual experimental behavior falls somewhere between these two limits, as indicated by the two solid lines typical of observed behavior. The difference between the actual experimental behavior and the behavior expected in Case 2 is the actual amount of ammonia oxidized to nitrate by the bed.

Figure 34 shows this nitrate production by Unit 1 in the experiment graphed in Figure 32, along with the plot for maximum possible concentration of nitrate. After prolonged starvation, the bed immediately recovers about 35% of its potential nitrifying ability. This indicates that at least a portion of the microflora survived the prolonged anaerobiosis. Assuming continued linearity of the developing nitrifying capacity, 4 to 5 days elapse before a state of complete nitrification is restored. This rate of development is probably tied to the growth rate of aerobic nitrifiers, as they replace numbers of their population which were rendered non-viable by the extended anaerobiosis.

Figure 35 shows a similar presentation of nitrate production data for Unit 2 after only a 40-hour period of anaerobiosis. A more vigorous return to nitrification is evidenced.

Gases Generated During System Operation

An analysis was made of the gases given off by the activated bed unit when operating in both the aerobic and anaerobic modes. Gas samples consisted of 500 cc grab samples taken from the head space entrapped above the liquid during anaerobic operation and from the discharge vent in the aerobic unit. This sampling was carried out specifically to determine if N_2O (nitrous oxide) was one of the by-products of denitrification.

A special analytical method was designed that utilized gas chromatography and mass spectrometry. This consisted of cryogenically concentrating each sample in a collection loop on the chromatograph, inserting the condensate into a 20 ft Porapak column set for automatic temperature programming, feeding the column effluent into a mass spectrometer introduction system and continuously monitoring the major ion fragment of N_2O with the electron multiplier analyzer system.

Standard samples of N_2O and an inert diluent were prepared and analyzed to ppb levels. Extrapolated sensitivities were verified by mass spectrometer calculations. With this procedure signals were observed from standards containing approximately 1 ppb. Reproducible quantitative measurements were achieved for samples containing 10 ppb of N_2O.

Multiple analysis of both gas samples were carried out. No N_2O was observed in either sample nor was CO, CH_4, or NO, present in any detectable quantities.

Efficiency of Nitrate Removal

As in the aerobic nitrification experiments, there exists a relationship in the anaerobic mode between denitrification rate, percent nitrogen converted, and mean residence time in the treatment unit. Although the experiments were not specifically designed to explore this aspect due to the time required for each run, there is enough data available to indicate trends and to arrive at tentative conclusions.

The most interesting relationship is the one between percent NO_3-N remaining in the denitrification unit as a function of residence time. This appears to be a more sensitive parameter than the rate-residence time relationship. In Figure 36 both these relationships are displayed. The denitrification rate appears to hold relatively constant over a broad range of residence time; as a consequence of constant rate, there is a dramatic response in process efficiency as residence time is increased. A 90% nitrate removal is achieved at about 475 minutes (8 hours).

The efficiency of the overall process can be estimated from the information in this report. In the aerobic stage about 85-95% of the incoming nitrogen can be accounted for as NO_3. This is fed into the denitrification stage wherein 85-95% can be reduced to nitrogen, accounting for an overall removal by the denitrification process of from 70 to 90% of the incoming total Kjeldahl nitrogen.

It is very informative at this point to compare Figure 36 with Figure 10 which displays similar data for the nitrification experiments. In Figure 10 we note that the rate of nitrification increases as residence time decreases. At short residence times the feed flow, and as a consequence the nutrient flow is increased. The microflora responds to the increased nutrients by increasing its growth or metabolic rate which results in all of the added nutrients being oxidized and as a result the percent nitrification and nutrient utilization efficiency remains high.

The events during denitrification present quite a different picture. As displayed in Figure 36 decreasing the residence time down to 200 minutes has almost no effect on the metabolic rate as displayed by the denitrification rate. Since the denitrification rate remains constant, the continual addition of more NO_3-N with the decreasing residence times means that there will be a marked sensitivity of process efficiency to changes in residence as is actually the case.

Under the conditions of the anaerobic mode used in our experiments altering the residence time would not effect the inflow of organic carbon - the carbon being already present in maximum concentrations as stored reserves. Since the nitrates are in excess it must

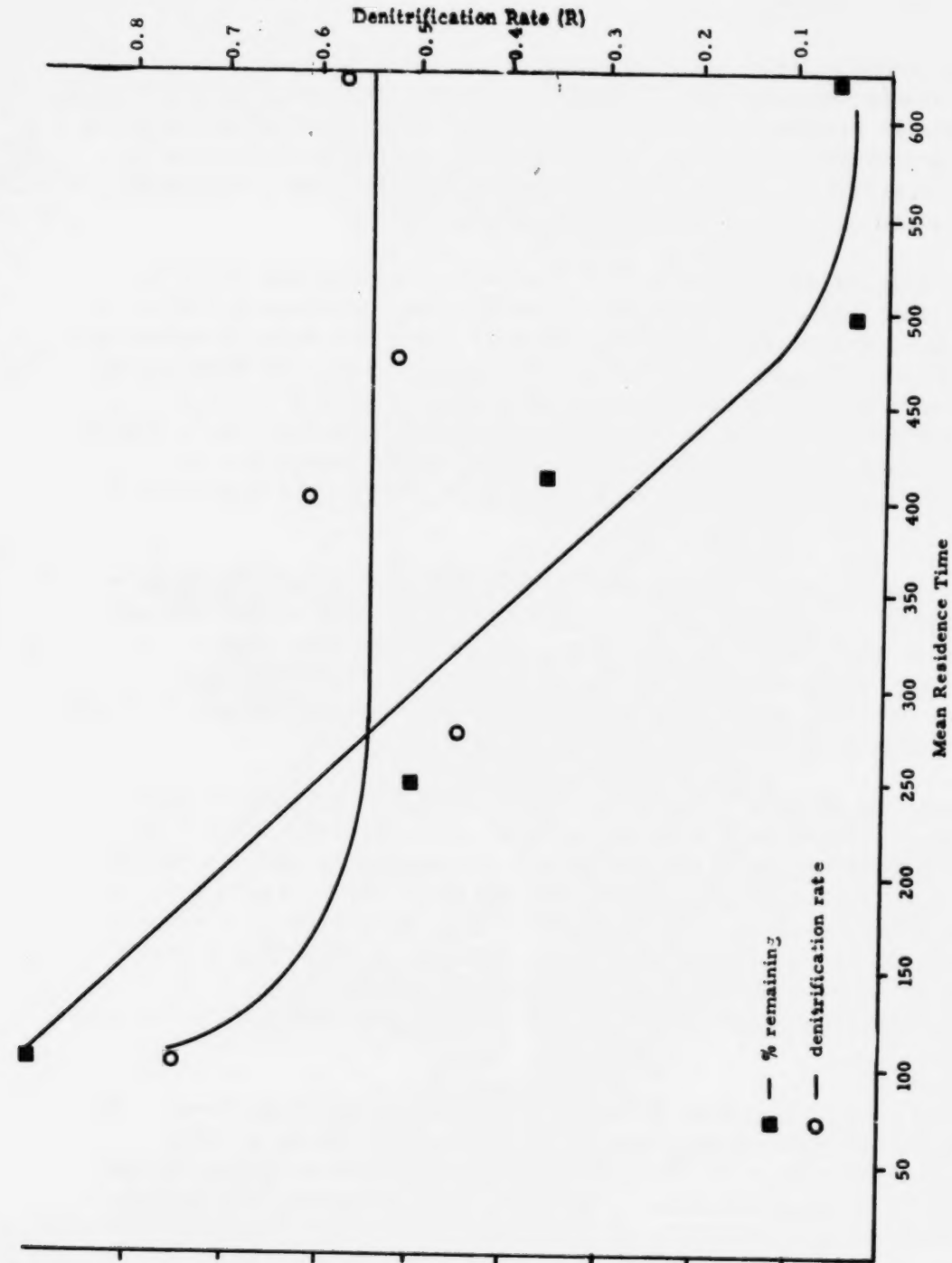


Figure 36. Influence of residence time on denitrification rate and nitrogen removal efficiency. These points represent a summary of the various phases of the 29°C denitrification runs. These are all averaged values for both units of the various runs discussed in this report. Denitrification rate (R) = mg/hr/g MLVS.

be concluded that the rate of denitrification is limited by the availability of the stored food reserves and in all probability this is organic carbon. Below 200 minutes the residual COD in the nitrified effluents may provide enough carbon to effect rates but the denitrification efficiency is very low. Thus, carbon limitation is the controlling parameter during denitrification. This shortcoming can be overcome for a given volume of effluent either by supplementing the influent with organic carbon or by increasing the mass (MLVS) of carbon containing microbial cells.

The residence time, denitrification efficiency, denitrification rate relationship needs further study. In this program, working with a primary effluent of a relatively fixed nitrogen concentration meant that there was a corresponding variation in organic load as flow rates were changed.

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IX. APPENDIX I

Analytical Techniques

(1) Chemical Oxygen Demand (COD). This was the principal test used to evaluate the efficiency of the oxidative process. The chemical method using the alternate procedure for a 10 ml sample size, as described in Standard Methods (1967), was used throughout this work. Samples were permitted to settle for 30 minutes in the cold before the 10 ml aliquot was removed.

(2) Total Kjeldahl Nitrogen (TKN). A micro Kjeldahl procedure was used employing an Aminco ^{R} Digestion and steam distillation system. Nitrogen was determined by nesslerization of the distillate. NH_4Cl was used as a standard.

(3) Ammonia Nitrogen. NaOH (18N) was added to the sample prior to steam distillation into boric acid. Ammonia nitrogen was determined by nesslerization of the distillate.

(4) Nitrates. The Brucine method, as described in Standard Methods was used for this analysis. Potassium nitrate was used as the standard.

(5) Nitrites. The packaged method of the Hach Chemical Co. was employed for this analysis. Comparative assay with other tests and the excellent reproducibility of the sodium nitrite standard showed this to be a reliable test.

(6) Mixed Liquor Suspended Solids (MLSS). The Activated Bed units were drained and the plastic rings containing the biomass were removed. Each ring and the sides of the vessel were scraped and rinsed with distilled water. The biomass suspension was placed in an oven at 105^{o}C and dried to constant weight (24-48 hrs). The total dry weight represented the biological solids contained in the treatment unit.

(7) Mixed Liquor Volatile Solids (MLVS). The dried solids remaining from the MLSS determination were carefully reweighed and then placed in a 600^{o}C oven for 24 hours. The loss in weight represents the volatile solids contained in the Activated Bed system.

(8) Phosphate. Phosphate was determined as the orthophosphate. The procedure Standard Methods (1967) was follwed except that an amino acid solution (Hach Chemical Co.) was used as the reducing agent.

(9) Protein. The colorimetric method of Lowry, et al (1951) was used. Bovine serum albumin was used as the standard.

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(10) Amino Acids. These were measured by determining the tyrosine, tryptophane content using a modification of the colorimetric method of Spiess (1957). Tyrosine was used as the standard. Proteins were first removed by precipitation with 5% trichloracetic acid. Total amino acids were calculated by assuming the tyrosine, tryptophane content as 5% of the total amino acids present.

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DERIVATION OF THE MATHEMATICAL MODEL

This derivation assumes: (1) that the nitrogen composition of the secondary effluent fed to the Activated bed units is rather constant and consists of 40-50 mg/l ammonia nitrogen (NH_3-N), and (2) that under anaerobic conditions a given biomass in one unit denitrifies at a constant rate, as ascertained in batch denitrification studies.

The following symbols are used:

y(t)	=	inorganic nitrogen in anaerobic unit (mg/ml)
R	=	constant rate at which biomass removes inorganic nitrogen (mg/min)
Q	=	volume of anaerobic system (ml)
F	=	feed flow of nitrified effluent to an anaerobic system (ml/min)
C(t)	=	$\left[NO_3\text{-}N\right]$ in aerobic unit (mg/ml)
V	=	aerobic system volume (ml)
f	=	feed flow of sewage to aerobic system (ml/min)

Also, let $F/Q = a$, $f/v = b$ and N_f = inorganic nitrogen in the feed.

Consider a system consisting of two Activated bed units, both operated in the aerobic mode (Figure 18 (a)). At time t = 0, one of the units (Unit 2) is switched to the anaerobic mode by turning off the air and feed flows, and simultaneously starting a metering pump to feed the highly nutrified liquor from Unit 1 into Unit 2 (Figure 18 (b)).

Examination of the figure reveals that there is one input flow and two exit flows of inorganic nitrogen from the anaerobic unit. The source of nitrogen is the incoming nitrified liquor from Unit 1 and this liquor contributes to the concentration of inorganic nitrogen in the system at any given time an amount of:

$$\frac{c(t) \cdot F}{Q} \cdot dt$$

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One source of nitrogen loss is the nitrate which leaves the unit via the overflow. This contribution to inorganic nitrogen change in the anaerobic unit is given by the term:

$$\frac{y(t) \cdot F}{Q} \cdot dt$$

The principal loss of nitrogen in the anaerobic unit is by means of denitrification. During continuous operation, this proceeds at some constant rate, R. The contribution to the change in inorganic nitrogen due to this loss is represented by:

$$\frac{R}{Q} \cdot dt$$

The combination of the terms for the nitrogen input with the two terms for nitrogen output gives equation (1) which describes incremental changes in nitrogen concentration in the anaerobic system.

$$dy(t) = \frac{c(t) \cdot F \cdot dt - y(t) \cdot F \cdot dt - R \cdot dt}{Q} \qquad (1)$$

Rearranging equation (1) gives us:

$$\frac{dy}{dt} + \frac{F}{Q} y = \frac{c(t)\, F}{Q} - \frac{R}{Q} \qquad (2)$$

where F, Q, and R are constants.

A solution for y(t) is obtained by noting that equation (2) fits the general form of the first order linear differential equation:

$$\frac{dy}{dt} + p(t)y = g(t) \qquad (3)$$

where p(t) and g(t) are any continuous functions of t. The general solution to equation (3) is obtained by use of the integrating factor, u(t), and is given by:

$$y(t) = \frac{1}{u(t)} \left[\left[\int^t u(s)g(s)ds + \text{const} \right] \right. \qquad (4)$$

where

$$u(t) = \exp \left[\int^t p(x)\, dx \right] \qquad (5)$$

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A comparison of equations (3) and (2) identified p(t) as $\frac{F}{Q}$, a constant and g(t) as c(t) $\frac{F}{Q}$ - $\frac{R}{Q}$, where F, Q, and R are constants. The only time dependence in g(t) occurs as c(t), the nitrogen concentration in the feed to the anaerobic unit, which is equal to the nitrogen concentration in the aerobic unit. Two cases for the time behavior of c(t) need to be considered. In the simplest case, c(t) is a constant, independent of time. This is the case represented by Figure 18 (b), where Unit 1, which has been aerobic for some time prior to the beginning of the experiment, remains aerobic, while Unit 2 goes anaerobic. The Unit 1 liquor maintains its steady-state nitrogen concentration, which is just N_f - NH_3-N assuming the feed flow to the aerobic unit has been judiciously chosen to give ~ 100% nitrification (which has been the case in all experimental work to date). Assuming then that c(t) = N_f, a constant for condition 18(b), the nitrogen concentration exiting the anaerobic unit, y(t), is given by the solution to the differential equation (substituting c(t) = N_f in equation 2):

$$\frac{dy}{dt} + \frac{F}{Q}\, y = \frac{F \cdot N_f}{Q} - \frac{R}{Q} \qquad (6)$$

From equations (4), (5), and (6):

$$y(t) = \frac{N_f F - R}{F} \left[1 - e^{-(F/Q)t}\right] + y(o) \left[e^{-(F/Q)t}\right]$$

where y(o) is a constant of integration which must be evaluated from the boundary condition at t = o. In this particular case, y(o) = N_f, since at t = o Unit 2 is switched from steady state aerobiosis to anaerobiosis. Thus, y(t) for Unit 2 from t_o to t_1 is:

$$y(t) = N_f \left[1 = e^{-(F/Q)t}\right] - \frac{R}{F} \left[1 - e^{-(F/Q)t}\right] + N_f \left[e^{-(F/Q)t}\right]$$

or simplifying and lettering F/Q = a

$$y(t) = N_f - \frac{R}{F} \left[1 - e^{-at}\right] \qquad (7)$$

Also, c(t) for Unit 1 from t_o to t_1 is:

$$c(t) = N_f \qquad (8)$$

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Now at time t_1 the units are switched (see Figure 18(c)), and a more complicated solution to the differential equation (equation 2) results since c(t) is no longer constant. In Unit 2 just at t_1, the liquor has been denitrified to a concentration $N_o^{t_1}$. At t_1, the air flow and feed are resumed to Unit 2, and, assuming that the biomass returns instantaneously to its previous completely nitrifying capacity, all available NH_3-N entering in the feed is immediately converted to nitrate. As the liquor that was in Unit 2 is gradually replaced by incoming primary effluent the nitrogen level rises to N_f. This Unit 2 liquor with nitrogen concentration rising to N_f provides the feed to Unit 1 (i.e., c(t) at flow F into Unit 1). Thus, to find y(t) in Unit 1 (from equations 2, 3, 4, and 5) a mathematical description of the nitrate concentration in Unit 2 (i.e., c(t)) is needed. A differential equation, similar in form to equation (2) when solved, yields:

$$\text{for } t_1 \text{ st } \text{st}_2$$

$$c(t) = (N_o^{t_1} - N_f)\, e^{-(f/V)(t-t_1)} + N_f$$

The equation is easier to perceive if the simplifications below are introduced.

$$f/V = b$$

$$(N_o^{t_1}, - N_f) = \Delta N^{t_1}$$

Thus,

$$c(t) = (\Delta N^{t_1})\, e^{-b(t-t_1)} + N_f \qquad (9)$$

Unit 1 at time t_1 is switched to anaerobiosis, with an initial concentration of nitrogen, $y(t_1) = N_f$, and is fed at rate F with liquor from Unit 2 at a concentration c(t) given by (9). Substituting (9) into (2) gives the differential equation for y(t), the nitrogen concentration in and exiting from Unit 1. This differential equation is solved in the same manner as in the simpler case where c(t) is constant, and the solution is, for t_1 st st$_2$ in Unit 1

$$y(t) = N_f - \frac{R}{F}\left[1 - e^{-a(t-t_1)}\right] + \frac{a(\Delta N^{t_1})}{a-b}\left[e^{-b(t-t_1)} - e^{-a(t-t_1)}\right] \qquad (10)$$

Further cycling of the units gives equations for c(t) and y(t) which are identical to (9) and (10) except for shifts in the time axis (i.e., t_2,

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t_3, t_5 ... are the switching times and appear in place of t_1 in equation (9) and (10)). For example, in the case of t_2 s t s t_3 the solutions are:

Unit 1 (aerobic):

$$c(t) = (\Delta N^{t_2}) \quad e^{-b(t-t_2)} \quad + N_f \tag{11}$$

Unit 2 (anaerobic):

$$y(t) = N_f - \frac{R}{F}\left[1 - e^{-a(t-t_2)}\right] + \frac{a(\Delta N^{t_2}}{a-b}\left[e^{-b(t-t_2)} - e^{-a(t-t_2)}\right] \tag{12}$$

Figure 37 illustrates the operations defined by these equations.

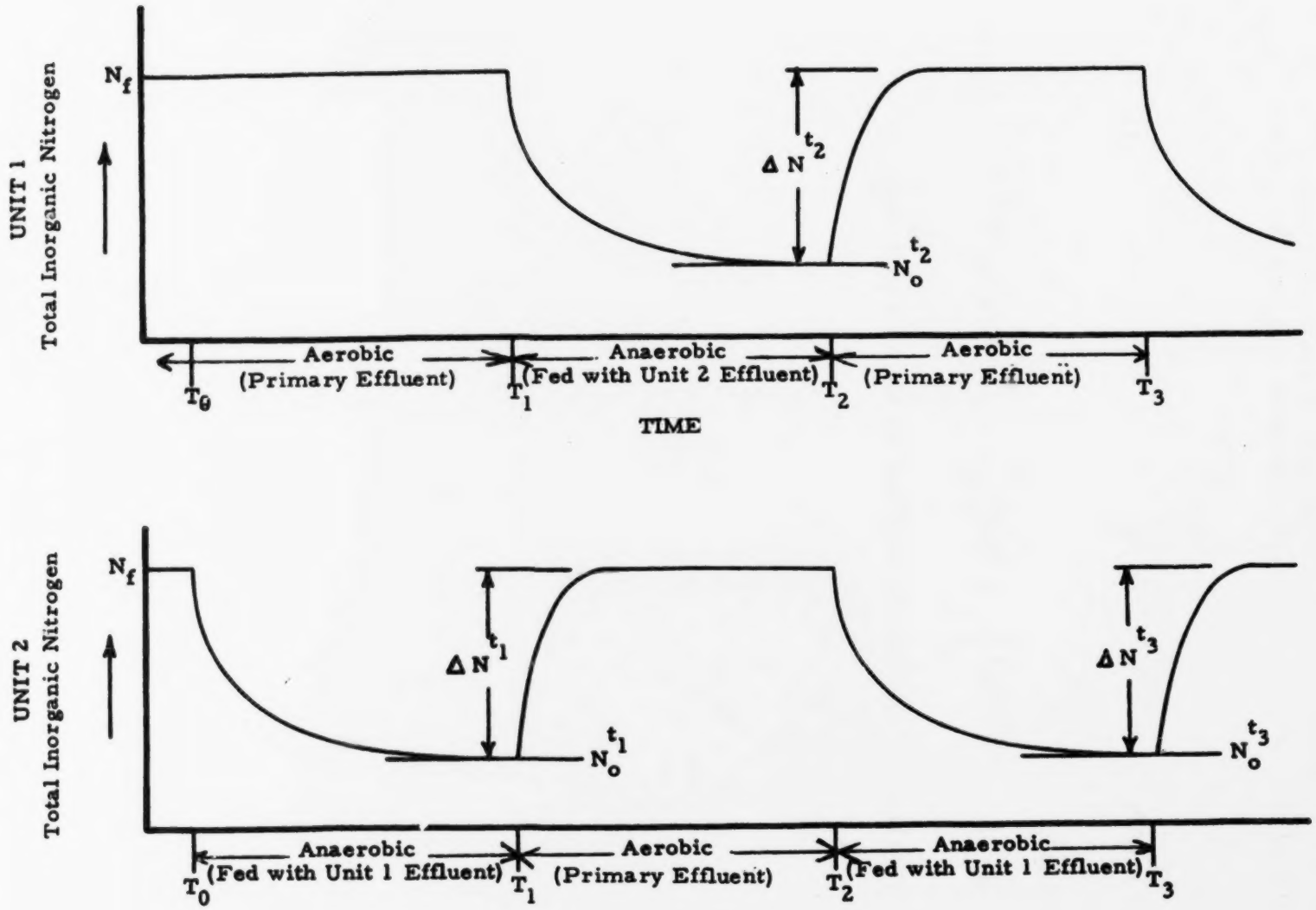

Figure 37. Graphical Representation of System Operation Defined by the Mathematical Model. Cycled aerobic and anaerobic operation, both units fed continuously.

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27 Abstract
A program to incorporate biological denitrification into a wastewater treatment system was undertaken with the objective of developing a process that depends exclusively on the carbon compounds contained in the wastewater to supply metabolic energy to the microflora. In the experimental program the incoming nitrogenous material was oxidized to nitrate in an aerobic phase and reduced to nitrogen gas in an anaerobic phase. Conditions for developing a nitrifying microflora were investigated using a primary wastewater effluent as feed. Flows into the system were varied to give a range of residence times. Anaerobic batch experiments were carried out to determine if stored reserves could support denitrification. Under appropriate conditions almost 100% of the nitrates could be reduced. The effluent from the aerobic unit served as the feed for the anaerobic process. At appropriate intervals this situation was reversed by switching the airflows and feed sources. Over 95% of the wastewater nitrogen in wastewater was removed. Nitrate-nitrogen removal rates ranged from 0.600 to 1.00 mg/hr/g MLVS. A mathematical model was developed which described the response to cycled aerobic-anaerobic operation. The alternating cycle approach was shown to be an effective method for removing nitrogen from wastewater.

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